



It is seen from table 1 that most of the white patients (92.1 per cent) developed malaria after the first transmission attempt and that 94.7 per cent became infected after one or more attempts. The Negroes became infected at a much lower rate after the first trial (28.6 per cent) and after multiple trials (31.4 per cent).

Of the 19 patients (8 white and 11 Negro) upon whom additional transmission attempts were made, the number of such trials were as follows:

- 4 (white—3; Negro—1) patients were infected on second trial.
- 1 (white—1; Negro—0) patient was infected on third trial.
- 6 (white—2; Negro—4) patients were not infected on second trial.
- 8 (white—2; Negro—6) patients were not infected on third trial.

TABLE 1

*Summary of all attempts to transmit foreign P. vivax to 186 patients*

	FIRST TRIAL	SUBSEQUENT TRIALS	TOTAL
White patients			
Tried.....	151	8*	151
Infected.....	139	4*	143
% infected.....	92.1	50.0	94.7
Negro patients			
Tried.....	35	11*	35
Infected.....	10	1*	11
% infected.....	31.4	9.1	31.4
Total patients			
Tried.....	186	19*	186
Infected.....	149	5*	154
% infected.....	80.1	26.3	77.4

\* Failures on first trial.

It is apparent that while additional trials infected 4 of the 8 white patients who failed on the first attempt, only one out of 11 Negroes was infected by repeated trials.

*Transmission of Foreign P. vivax According to Origin of the Malarias.* The origins of the malarias transmitted are shown in table 2.

There was less difference in the transmission rates of the malarias from the Pacific and those from the Mediterranean areas than between different places within each of these areas.

*Transmission of Different Strains.* For convenience, each relapsing case of malaria studied in returned troops was arbitrarily designated as a different strain and assigned a number.

In the transmission attempts, 43 strains were involved of which 34 were transmitted. These data are presented in table 3.

Most of the strains from every area infected white patients. All of the 11





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routes controlled by measurement of the specific gravity of the blood and good medical care are the most valuable therapeutic procedures. The general behavior of the disease suggests that dehydration and the depletion of salts are of far greater importance than toxemia, in the usual sense of the term, in the causation of late symptoms and death.

Other than a prompt reduction of the numbers of vibrios in the stools and slight shortening of the attacks, there is no evidence that streptomycin given orally or parenterally influences the course of the disease. Strains of *V. comma* vary greatly in their resistance to streptomycin in vitro.

Since it is doubtful that in all instances the public water supply is the chief source of cholera or of its perpetuation, and because infection is often conveyed by numerous factors of personal contact, the best measures for the prevention of cholera rest in the universal adoption of many simple hygienic measures. The control of cholera is more of an economic problem than a medical one.

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# A STUDY OF HOOKWORM INFECTION IN NAVY AND MARINE PERSONNEL ON GUAM

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Two questions were of interest concerning shore-based Navy personnel and marines in relation to hookworm in the Pacific Islands: How much hookworm infection were they contracting under garrison or combat conditions? Did *Ancylostoma duodenale* form a part of it?

During early 1945 examination of native Guamanians revealed a 90 per cent incidence of hookworm, with high individual worm burdens, predominantly of *Ancylostoma*. Temperature and moisture (both humidity and rainfall) on Guam are ideal for the propagation of hookworm in its external phases. With factors both parasitic and environmental present in such degree as to represent a measureable threat of infection, the use of Guam as a base of operations prompted us to ascertain to what degree hookworm and other intestinal parasites were present or establishing themselves in service personnel on the island.

The service men we examined fall into two natural groups. Group I, garrison forces, was composed of men most of whom had come to Guam soon after the American reoccupation in July–August 1944, and had not been further west. Examination of them 5 to 9 months after their arrival there showed a low parasitic index. The hookworm infections were primarily *Necator americanus* and, from collateral data, were interpreted as having been mainly contracted at home. Group II was composed of marines (artillery), who had been on Guam for 5 to 7 months following combat exposure in the Philippines during the rainy season. In them the parasitic index was high, especially with regard to hookworm. Clinical hookworm disease with anemia was not encountered, but some heavy infections were revealed by egg count and verified by worm count following treatment. *Ancylostoma duodenale* was demonstrated in both Groups I and II, and especially in the latter. There was also evidence that a certain amount of reinfection was taking place on Guam.

## METHODS

*Sampling.*—After preliminary announcement at muster concerning the purpose of the survey, fecal specimens were routinely obtained by having the corpsmen at the heads for early morning collections. To supplement this method, men, whose names were chosen by a method of random sampling brought specimens directly to the laboratory. These methods of collection furnished specimens which could be reliably attached to the names of the donors. Al-

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which are common in regions where hookworm is endemic, readily produce infection when such habits are practiced in tropical islands in the Pacific. The presence of 2.7 per cent infection in Group I men from outside the southern States may be evidence of this. This factor is presumably present in Group II as well, partially obscured by the number of new infections. On the other hand, the new infections in both groups may bespeak to some extent ancylostomiasis contracted through fomites, as recently demonstrated by Loughlin and Stoll (1946).

*Worm burdens.*—There are two methods of obtaining data on the worm burdens of infected persons. One is indirectly by means of the number of eggs per unit quantity of feces. The other is directly by means of worm counts after treatment.

In table 5 are presented the original data on 33 cases treated with tetrachloroethylene. These are listed in order of increasing egg counts, and certain egg count classes have their average worm counts derived to indicate the comparative meaning of such classes. (It may be mentioned that in table 5 the data are presented to indicate their maximum degree of variability. Thus, only a single egg count, the first of the case, is used to classify it; no post-treatment egg counts are given to show the efficiency of treatment; and the worm counts are for the minimum period of one day, whereas some worms continue to be dislodged for 2, 3, or more days after vermifuge.)

The information in table 5 is interesting from several angles. We use it first to permit a classification of egg counts as a reflection, in general, of worm burdens. The relationship is not a linear one, but holds as a general correlation, despite the presence of mixed *Necator* and *Ancylostoma* infections and despite examination for worms for only 24 hours after treatment. Thus, no case with "less than 1000" eggs per gram of feces showed as many as 10 worms, whereas cases with more than 10,000 eggs per gram average over 200 worms. On the basis of these data we combine our egg count positive cases for further analysis into four simple classes: Those showing less than 1000 eggs per gram of feces as minimal positives; those having from 1100 to 5000 as light; those with from 5100 to 10,000 as moderate; those having over 10,000 eggs per gram as heavy.

In table 6, the 71 positive cases in Group I and the 253 in Group II are arranged so as to show their distribution in the minimal, light, moderate and heavy egg count classes. It is immediately apparent that the bulk of the positives in Group I and Group II are minimal. Most of the remaining are light cases. However, 7.0 per cent of Group I positives and 6.4 per cent of Group II positives are moderate and heavy infections.

Also shown in table 6 are the percentages of all men examined who fall into these classes. In Group I, 0.4 per cent of the men show moderate and heavy infections, while in Group II, 2.2 per cent fall into these classes.

From this analysis it is clear that the exposure to hookworm of the marines on Leyte resulted primarily in minimal and light infections. At the same time, the number of moderate and heavy cases in Leyte-exposed marines is over 5 times (Group I, 0.4 per cent; Group II, 2.2 per cent) that of men typified by garrison forces on Guam.

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cates that we are dealing primarily with hookworm infections contracted in the United States. In general, these infections have been light. Even when more than 50 or 100 worms were present, the hosts have not presented clinical symptoms, and, in the absence of routine survey, would probably have escaped detection and treatment.

Not all infections in Group I can be accounted for in this way. Men from outside the southern States are also infected. In some instances, these infections may have been contracted in the south during periods of military training. The presence of even a few *Ancylostoma* in post-treatment worm counts indicates that they are evidently also being contracted on Guam with its overwhelmingly large *Ancylostoma* index in natives.

*Group II.*—The military operation on Leyte, October–December 1944, occurred on an island with a record of considerable hookworm. An incidence of 93 per cent was reported by Manalang (1925) during a hookworm campaign there, and a decade later Tubangui *et al.* (1935) found 75 per cent of 169 persons positive by dilution counting, with an average of 3850 EPG. Except for Cotabato on Mindanao, this showing on Leyte was the most severe hookworm infection of the 10 areas of the archipelago which they studied. Concerning the presence of *Ancylostoma*, Leach *et al.* (1923) working at Bilibid prison, who worm counted 100 men after treatment, found 17.2 per cent of the 3539 hookworms recovered were *A. duodenale*. Nine of the prisoners were from Leyte. These 9 averaged 101 hookworms per individual, with 13.5 per cent *Ancylostoma*.

Of the men examined by us who took part in the Leyte campaign, approximately 1 in 3 were infected by hookworm, and 3 in 10 had *Ancylostoma*. Men from southern States showed a larger number of positives, but the picture in general is one of widespread new exposure to both *Ancylostoma* and *Necator*, superimposed in certain southerners on some *Necator* infection already present. Most of the infections are light, with enough heavier infections to indicate a strong possibility of successfully introducing the *A. duodenale* component to the southern part of the United States by returning service men.

Our data have been obtained from men living under the strenuous conditions of war. They demonstrate that the sanitary barriers devised by the Naval Medical Corps as applicable against hookworm are good, but that a measurable degree of breakthrough occurs in terms of new hookworm infections acquired. In peacetime less efficiency of the sanitary barriers can be anticipated. An increased rate of newly acquired hookworms is thus predictable.

#### SUMMARY

1. The examination of 1241 shore-based Navy personnel on Guam (Group I) showed a hookworm incidence of 5.7 per cent. Most of these infections were light, and many were evidently residual infections from the southern part of the United States. Some heavy infections were found, however, and some *Ancylostoma* were seen. Both facts are indications that some of the worms were acquired on Guam.

2. The examination of 742 Leyte-returned marines (Group II) showed a hook-



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experiments. Particulars concerning the material used and method of exposure are summarized in table 2. As shown there, the highest rate of infection was obtained in a group of 7 laboratory-reared snails of Lot C, in which 4 snails became infected. In this case, and also in the other two experiments with laboratory-reared specimens of La. C origin, the snails were juveniles of varying size; in the two experiments with La. K snails, in each of which 1 specimen was infected, the snails were adult.

TABLE 2

*Origin of material and method of exposure in cases of infection of Tropicorbis*

LOT	AGE	NO. SNAILS EXPOSED	NO. SNAILS POSITIVE	ORIGIN OF SCHISTOSOME EGGS	METHOD OF EXPOSURE
La. C	Juvenile	6	1	Hamster liver	As group. Numerous miracidia, repeated on 4th and 8th days.
La. C	Juvenile	6	2	Hamster liver	As group. Numerous miracidia on 5 successive days.
La. C	Juvenile	7	4	Hamster liver Monkey feces	As group. Numerous miracidia, repeated on 7th and 12th days.
La. K	Adult	4	1	Hamster liver	One time. Individually, 3 to 6 miracidia per snail.
La. K	Adult	4	1	Mouse intestine	As group. Ten miracidia 2 times, 1 day intervening, after failure of earlier individual exposure.

#### DURATION OF INFECTION IN TROPICORBIS AND INFECTIVITY OF CERCARIAE

The earliest emergence of cercariae of *S. mansoni* noted from experimental infection of *Tropicorbis* was on the 28th day after exposure to miracidia. The number of cercariae was small at first, 3 to 5 daily, but gradually increased; the maximum daily output of about 50 cercariae was reached after about two weeks. As to duration of infection one of the infected laboratory-reared *Tropicorbis* shed cercariae for 107 days and then died; on dissection after death, numerous sporocysts in various stages of development and fully developed cercariae were found.

A feature of particular interest was the superimposing of an experimental *S. mansoni* infection on an already existing infection with another species of trematode. Seven days after a wild specimen from lot La. K was exposed individually to from 3 to 6 miracidia, there were observed in the water large numbers of a split-tail cercaria notably different in appearance from cercariae of *S. mansoni* and having the characteristics of a strigeid; 31 days after the experimental exposure, cercariae of *S. mansoni* were also noted, and the two species continued to appear for a period of 23 days, at the end of which time the snail died and yielded on dissection two species of sporocysts and cercariae. Only the *S. mansoni* cercariae proved infective for mice.

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desired level. Then the pH was adjusted with N/10 NaOH to 7.0 using bromthymol blue as indicator. The pH had to be read instantly because of rapid fading of the indicator.

4. The cyst suspension was poured into the prepared water, a rubber stopper placed in the flask and the mixture shaken vigorously. Shaking was repeated at intervals throughout the contact periods.

5. 10 minutes later the residual chlorine in the mixture was determined by the orthotolidine method. Values up to 2 parts per million were measured using the test set supplied for this purpose by the Army. Higher concentrations were measured using the color standards of Muer and Hale appearing in "Standard Methods for the Examination of Water and Sewage", 8th edition (published by the United States Public Health Service).

6. At the end of the contact period a sample of the water was withdrawn for determination of the pH and residual chlorine. The chlorine in the flask was then neutralized by the addition of sufficient sodium thiosulfate with vigorous shaking.

7. The flask was emptied into a tall graduate and left to stand 4 to 5 hours while the cysts settled.

8. The lower 150 cc. of fluid were siphoned off and the cysts further concentrated by centrifugation. All but several cubic centimeters of the supernatant was drawn off.

9. The sediment was mixed with the small amount of remaining water and pipetted into tubes of either one or both kinds of culture medium. At the same time each tube was seeded with a half cc. of a bacterial suspension originally isolated from a successful culture of *E. histolytica*, but proved to be ameba-free by microscopic examination of repeated subcultures. No starch was added to these tubes because it was believed that excystation proceeded better in the absence of starch.

10. After 24 hours incubation at 38°C the sediment in each culture tube was transplanted to 2 fresh tubes of medium.

11. 48 hours later the sediment of these first subcultures was examined for amebae. If negative, transfer of one half cubic centimeter of sediment from each tube was again made to 2 fresh tubes of medium.

12. Forty-eight and 72 hours later a careful microscopic examination was made of the sediment in each tube seeded as described in Step 11 above, and also the cultures from which these seedings were made. If the original inoculum from the chlorinated water contained viable cysts, some trophozoites in one or more of the tubes were observable at these times. The concentration of amebae was in some cases enhanced by further transplants.

Only one or two analyses for total nitrogen were made (by the Chemistry section of the Laboratory) for a particular dilution of the cyst concentrate. It was assumed that the total nitrogen content would be approximately the same in all mixtures of a particular cyst concentrate and water from the same tank providing the proportions were the same.

# INTERNATIONAL APPRAISAL OF RESEARCH IN TROPICAL MEDICINE<sup>1</sup>

PRESIDENTIAL ADDRESS, 1945, AMERICAN ACADEMY OF TROPICAL MEDICINE

MARK F. BOYD

Members of the American Academy of Tropical Medicine, ladies and guests: I am deeply appreciative of the honor you bestowed upon me through elevation to your presidency in the current year, and wish to express my thanks for the distinction. I found the routine duties of the office to be far from onerous, but realization of my inadequacy to meet fittingly the ephemeral climax of a presidential address has given rise to sleepless nights. I know that on such an occasion as the present, a speaker has his auditors at a disadvantage during this brief exercise of his prerogatives, and I greatly hope that you, sufficiently surfeited by this banquet, will have acquired a complaisant mood, judging my remarks in a spirit of leniency, permitting appreciation of brevity to outweigh its very apparent short-comings.

When the Academy's committee on the award of the Theobald Smith Medal, announced their selection of Dr. Charles M. Wenyon, the distinguished English protozoologist, to be the recipient of this year's award of the Medal, it was anticipated that Dr. Wenyon could receive the presentation in person at this meeting, making the occasion memorable to us by a fitting address. It was with regret that we learned of Dr. Wenyon's inability to be present. Owing to the condition of his health, his physician has forbidden foreign travel. Fortunately the American Ambassador to the Court of Saint James, the Honorable John Winant, has cordially agreed to make the presentation at a meeting of the Royal Society of Tropical Medicine to be held at Manson House on December 13th. The Medal and Certificate of award have been transmitted to Mr. Winant through the good offices of the State Department. Should any members of the American Academy of Tropical Medicine or the American Society of Tropical Medicine be in London on this date, it is hoped they will participate through attendance at this meeting.

We are meeting at the close of the most catastrophic period in the history of the human race, a catastrophe caused not by upheavals of nature beyond human control, but the result of forces created and unleashed by human agency. We should be humbly thankful that in this contest, and as the result of indescribable exertions and sacrifices in blood and treasure, victory has been attained in collaboration with our allies.

Most of those present on this occasion are either directly interested in extending the domain of science, or in the application of the newly acquired knowledge. To us scientific research is a matter of vital interest, despite the circumstance that during the past century the radius of human knowledge has been

<sup>1</sup>Presented at the Annual Meeting of the American Academy of Tropical Medicine, Cincinnati, Ohio, Nov. 14, 1945.

# THE PULMONARY MANIFESTATIONS OF SCHISTOSOMIASIS CAUSED BY SCHISTOSOMA JAPONICUM

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The purpose of this paper is to present two cases of Schistosomiasis caused by *Schistosoma japonicum* in which prominent pulmonary manifestations were present.

## CASE REPORTS

*Case 1.* This 19 year old white soldier entered the hospital on 30 Dec. 1944, complaining of chills, fever, and supra-orbital headache of two days duration. He had arrived on Leyte, Philippine Islands, on 20 Oct. 1944, and had on several occasions in the interim bathed in a fresh-water stream. At the time of admission to the hospital his temperature was 100.8°F., pulse rate 88, and respiratory rate 18. No abnormal physical findings were elicited, and the case was considered to be one of dengue.

The blood count showed 11,600 white cells/cmm., of which 87% were segmented neutrophils and 13% lymphocytes. Instead of improving the patient continued to run fever of 100 to 104 and appeared to be acutely ill. On 2 Jan. 1945 the patient was coughing slightly and crepitant rales were recorded in both lung fields. The rales persisted without signs of consolidation, and on 4 Jan. a portable chest plate was made; the film was technically poor but showed some soft infiltration in the left base. Sulfadiazine was started in the usual dosage. Malaria smears were negative. The white blood count rose to 19,250 with 93% neutrophils on 5 Jan. Throughout this period the patient had been constipated and had very poor appetite. On 7 Jan. the white blood count rose to 21,850, of which 25% were eosinophils. The following day examination of a stool specimen obtained by enema revealed ova of *Necator americanus*, cysts and pre-cysts of *Entameba histolytica*, and ova of *Schistosoma japonicum*. At this time the patient was still acutely ill. His temperature had been 103 to 104 for the last five days. He had marked anorexia, constipation and abdominal distention. The spleen was questionably palpable and the liver was not felt. There were scattered rales throughout the chest and the patient had a non-productive cough. There had been no noticeable response to sulfadiazine, and the drug was discontinued. On 9 Jan. the administration of tartar emetic (potassium antimony tartrate) in treatment of the schistosomiasis was begun. This was given intravenously as a 1% solution in distilled water. The initial dose was 0.03 Gm., and on every second day thereafter he received 0.06, 0.09, 0.12, and 0.15 Gm. respectively; then 0.15 Gm. every second day. The intended therapeutic course included ten injections totaling 1.2 Gm. of tartar emetic.

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extending with a constantly increasing momentum to such distances that scientists can no longer be familiar with all disciplines, but find themselves fully occupied in a small segment of the circumference with little or no acquaintance with their conferees who are working with similar devotion in other segments.

I am sure that most if not all of us engaged in these pursuits, labor with either the avowed or secret hope that our efforts, no matter how humble, may contribute to the well-being of mankind. Admitting this laudable motivation, we should view with inexpressible anguish and shame the degree to which science has contributed to the present desolation of the world, and has been prostituted to the injury of the human race. There is no novelty in this situation, warfare has ever been prompt to exploit science since the bow and arrow supplanted the sling shot, and guns supplanted the crossbow. The extent to which advances in physics and physical chemistry have contributed to this situation, and in a vicious cycle have in turn been stimulated by military competition is appalling, and our colleagues in those fields of science must realize they have assumed responsibilities of a gravity perhaps never before borne by human beings, and have yet to demonstrate their capacity for the very grave task. Obviously in certain aspects of these fields, scientific research has outstripped human capacity to assimilate this knowledge, and has created a Frankenstein which if again unleashed will likely destroy our civilization.

Interested as we are in medicine and related fields, we can view with satisfaction and gratitude the circumstance that in our segments of knowledge, the exigencies of war resulted in progress and extension of knowledge in many limited fields, which promoted the welfare of our forces but contributed materially to the success of our arms without acquiring the stigma of prostitution to the ends of combat. Their continued and widened application will materially contribute to the healing of the deep wounds civilization has received.

The military necessities of the war period stimulated extensive research in tropical medicine and related fields in the United States and allied countries, resulting in numerous and significant discoveries. They also stimulated an unparalleled interest in the subject in the medical profession and among the laity, which is expressed by the noteworthy increases in membership of both the American Society of Tropical Medicine and the National Malaria Society. They required that millions of Americans of the armed services be stationed abroad on tropical assignments for extended periods, so that multitudes, to whom the word tropics formerly only vaguely expressed a region of perpetual summer, now are personally acquainted with both the charms and drawbacks of life in these regions. The charms may induce many to seek permanent employment and residence in the tropics, the drawbacks may result in chronically impaired health for many; and the ailments of veterans of these campaigns will for years offer perplexities to any uninstructed physicians who may be consulted about them. While the return of peace may result in a recession of interest in this subject, it is likely that, for the reasons enumerated, it will actually witness the maintenance of a higher level of interest than prevailed prior to the war. The phenomenal growth of international air travel will further contribute to the sustenance, if not the intensification, of this interest.

The success of the rectal mucosa biopsy technique in proving the infection present in 100 per cent of the untreated cases of schistosomiasis against 40 and 62 per cent, respectively, obtained through repeated examinations by the DeRivas method and the intradermal test, cannot be over-emphasized. The clinician has, at long last, a means at hand with which to solve and to study many aspects of this condition. The technique is an entirely safe one. Its execution takes only a few minutes and needs very little time to master. Complaints may be raised to the effect that the patient is exposed to some danger, or that he may be subjected to unnecessary mental and physical strain, but the experience of the writers has not proved this to be the case. In addition, the complete reliability of the method far outweighs any of the probable handicaps mentioned.

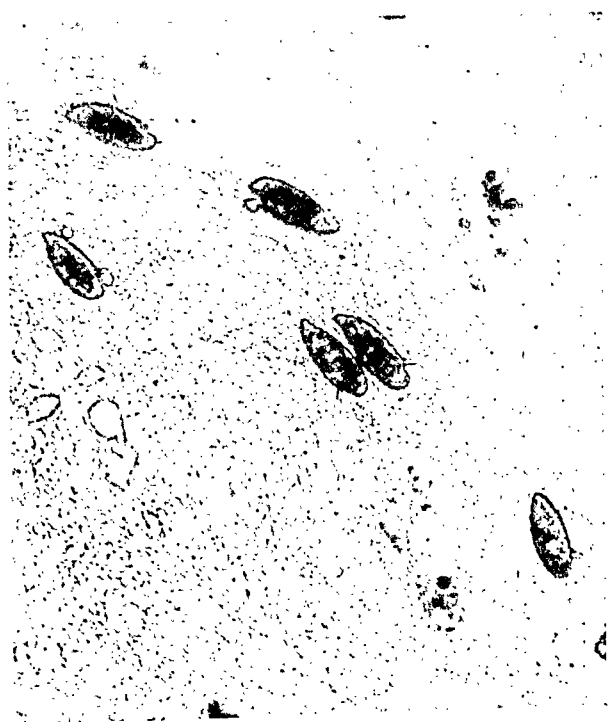


FIG. 1. DEAD *S. MANSONI* EGGS. MOST COMMON FORM ENCOUNTERED IN RECTAL MUCOSA BIOPSIES

The writers have been immensely gratified on several occasions by proving the existence of *Schistosoma mansoni* in patients whose stools had been repeatedly negative, when fecal samples were examined by the acid-concentration technique.

During the course of the present study, it was discovered that a rectal mucosa biopsy is not only of help in the diagnosis of the disease but also in the possible elucidation of many problems, as yet obscure, in schistosomiasis. The eggs, as they appear in the fresh tissue, may be divided, according to their character, into three major types.

By far the most common is the egg that appears heavy and black under the microscope (fig. 1). The second most common is that one which the writers have called the empty shell (fig. 2), since the yellow, chitinized remnant of the shell is all that remains of the egg. These eggs appear oftentimes collapsed,



The prompt and energetic application of this dearly purchased knowledge will go far to alleviate much of the widespread misery and wretchedness intensified by the war. Effective application requires rapid and extensive diffusion, neither of which in adequate degree can be attained through the ordinary channels of professional and scientific literature. The requisite initial impetus can best be attained by an international reunion of those who have contributed to these advances, with others qualified to disseminate and apply this knowledge. Such an international gathering of scientists will further materially contribute to better international understanding and cooperation, so essential to an enduring peace.

The Council of the Academy, convinced that these aims can best be attained by an International Congress of Tropical Medicine and Malaria, have recommended to the favorable consideration of the academy at the business session, the adoption of a resolution inviting the American Society of Tropical Medicine, the National Malaria Society, the Southern Medical Association, the American Society of Parasitologists, the American Medical Association, the American College of Physicians, the American Association for the Advancement of Science, and the Section on Medical Science of the National Research Council, to join with the American Academy of Tropical Medicine, in adopting a resolution petitioning the State Department of the United States government officially to sponsor and invite international participation in such a gathering at as early a date as may be regarded as opportune. The suggested resolution would also direct the president of each organization to appoint a representative of that organization to a general committee, available to assist the State Department in an executive capacity in developing these plans, and in promoting and holding such a congress.

The last international congress in this field was held during 1938 in Amsterdam, under the patronage of the Dutch government. It was well planned, the Dutch hospitality was gracious, wholehearted and flawless, but many of those participating, including your president among their number, were distracted by the ominous reverberations of the Munich conference, and attendance suffered as a consequence. Most of you can recall the effect of the events of 1939 on the Congress of Microbiology held in New York City in that year.

The Amsterdam Congress was the first world congress jointly held in the fields of tropical medicine and malaria, although it was the third of each, their predecessors having been held independently. Since inception the Malaria Congress had maintained a permanent intercongressional committee, and although nothing comparable existed for the Tropical Medicine Congress, the executive committee of the Third Congress of Tropical Medicine was designated to serve as an interim commission until the organization of the Fourth Congress.

As far back as the Second Congress of Tropical Medicine, held in Cairo in 1928, desire was expressed to hold the Fourth Congress in some American country. Following the Second Malaria Congress in Algiers, it was intimated that the malariologists would be receptive to an invitation from the United States for the Third Congress. But at that time many in the United States felt that adequate support could not be secured and the committee developed plans to hold the

Third Malaria Congress in Madrid in 1935. These plans were frustrated by the outbreak of civil disorder in Spain, which lead ultimately to the joint meetings in Amsterdam. The interim committee of the Third Congress was charged with the task of contacting the countries of America to ascertain which would be willing to exercise the privilege of priority with regard to the Fourth Congress.

If this proposal meets with a favorable reception from those organizations to which submitted, it would appear to me highly desirable to preserve the chain of continuity by recognizing the interim committees of the two congresses, expressing to them the desire of the United States to be host to a Fourth Joint Congress of Tropical Medicine and Malaria, and requesting their recognition of the proposed intersociety committee as the executive committee for the Fourth Congress.

It might be found practicable to organize the Congress on the basis of four membership classes, viz

- a) Sustaining memberships, available to commercial organizations;
- b) Official delegates or members, designated by their governments with official credentials;
- c) Unofficial (but professional) members; and
- d) Associate members (non-professional).

This is mentioned not with any desire to suggest to any future executive committee the direction of its labors, but merely to mention at the moment what would appear to be practical *modus operandi*.

Prevailing circumstances perhaps will not have ameliorated sufficiently to make 1946 an opportune year for the meeting, and selection of that year would probably not give sufficient time to lay the ground work and perfect plans in sufficient detail. However considering the labor involved, it is probably not too soon to lay plans for 1947.

In conclusion I wish to express the hope that you will agree to the proposal for the solicitation of the support of related organizations and the State Department to this project, with the conviction that the need is great and the time is propitious.

# LESSONS IN MALARIOLOGY FROM WORLD WAR II<sup>1</sup>

THE CHARLES FRANKLIN CRAIG LECTURE, 1945

PAUL F. RUSSELL<sup>2</sup>

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Man's net losses from World War II are so enormous that it would be illogical indeed to refer to war-produced scientific advances as dividends or to point to them with thoughtless pride. Rather, such progress constitutes salvage which, to be sure, sometimes has considerable value because conditions of war while they rarely permit classical research do present an urgency which demands, and often obtains, quick answers to difficult problems. New lessons are learned and others re-learned, painfully and at great expense.

Military requirements during the past six years undoubtedly stimulated malariology and it is fitting to discuss the subject in a lecture which honors Colonel Craig,—a military surgeon who, during his years of active service, was the Army's foremost malariologist. His textbook on the malarial fevers summarized the subject up to 1900 and may still be read with great profit. Just as Craig made plain the fact that the rapid progress of the 1890's and early 1900's produced no malaria panacea, so one would emphasize now that no magical wand for malaria control came out of World War II (although one might think so from exuberant press releases). On the contrary, military experience taught once more that the prevention of malaria is neither automatic nor simple but is compounded of law and persuasion, organization and training, supplies and technical application. The lesson should have been well-known, but it had to be learned again. It has not been found possible to control malaria by directives, or to devise miraculous weapons, yet there have been notable developments in the parasitology, entomology, clinical aspects, and prophylaxis of this disease during World War II.

## PARASITOLOGY

In the parasitology of malaria, the greatest advances were along three lines, (a) towards better understanding of life history and biochemistry of the parasite; (b) in development of technique for experimental malaria; and (c) in cultivation of plasmodia.

Exoerythrocytic forms have not yet been satisfactorily demonstrated in mammalian malaria but Huff (9) and others have shown that in at least six species of avian malaria there are unpigmented stages of the plasmodia in cells other than erythrocytes. Although such stages remain undisclosed in man, the recent energetic search for them has taught lessons in tissue parasitism which will

<sup>1</sup> Presented at the fifty-first annual meeting of The American Society of Tropical Medicine, Cincinnati, Ohio, November 13-15, 1945.

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eventually lead to more effective antimalarial drugs because hidden aspects of the plasmodium have been exposed to the light of direct experiments in therapeutics.

Not only have obscure phases of parasite development been revealed but advances have also been made in the biochemistry of plasmodia, and cells infected by them. In fact, one of the important lessons of the war years has been that there is great advantage in close cooperation between parasitologist and biochemist.

Studies of exoerythrocytic forms and in biochemistry were but parts of a great expansion of experimental malaria, guided by the Committee on Medical Research and financed by the Office of Scientific Research and Development. New methods and improved techniques have been developed. Now, for example, the antimalarial potentialities of large numbers of drugs can be evaluated much more rapidly and effectively than ever before.

Another advance has been in the cultivation of plasmodia. Here also progress has been more rapid because of the backing of such war-stimulated agencies as the Medical Research Council in Great Britain and the National Research Council in this country. Hawking (8) in England contrived a tissue culture method which is useful in growing exoerythrocytic stages of *P. gallinaceum*. Ball, Geiman and associates (1) at Harvard devised a technique for the culture *in vitro* of erythrocytic forms of *P. knowlesi*. The latter method has value not only in studying physiology and chemistry of plasmodia but also in several other respects, including assistance in explaining how drugs act to cure malaria. The effect of antimalarials *in vitro* in these cultures closely parallels that seen *in vivo*.

Other points could be made. For instance, strain differences within species of plasmodia became clearer during the war. Diagnosis by blood film was again demonstrated to be a procedure requiring well-trained and supervised personnel. Facilities in this country for such laboratory help are now better developed than they were before the war. Serological diagnostic tests received much study and while not yet entirely practical are nevertheless becoming useful. All-in-all, considerable progress in the parasitology of malaria occurred during World War II.

#### ENTOMOLOGY

The greatest advances in entomological phases of malariology have been along two lines; (a) in developing the taxonomy of certain anopheline groups, especially in the southwest Pacific; and (b) in collecting scattered data to form world keys for identification of *Anopheles* species in any region, however remote.

Allied Forces have been deployed all over the world. This fact has stimulated a broad increase in knowledge of mosquitoes. For example, never before have there been such comprehensive taxonomic collections as are now in Washington at the National Museum and the Army Medical School. Never have keys been so extensive and practical in their scope. Moreover, much has been learned about the principal malaria vectors of the world so that species control, whether by sanitation or eradication, has a much firmer foundation.

In connection with entomology, mention should also be made of the prevention

of inadvertent transportation of malaria vectors from one area to another. Unbelievable expansion of Allied air forces, and of shipping, tremendously increased this hazard, so clearly illustrated by the disastrous *gambiae* invasion of Brazil in 1930. It is remarkable that, although some vectors have extended their pre-war range, thus far none appears to have been carried across sea or ocean in numbers sufficient for colonizing. Newer methods of spraying planes and ships, and better control of airfields and seaports have been important lessons from World War II.

#### CLINICAL MALARIA

In the field of clinical malaria the chief lessons have been three in number. First, better methods have been formulated for using atabrine and plasmochin. Secondly, totaquine has again been found to be a good substitute for quinine sulfate. Thirdly, the lesson has been driven home, as many times before, that vivax malaria is a relapsing disease.

Most of the world's cinchona plantations and alkaloid factories were captured by Germany and Japan, so that the Allies were faced in 1932 with an irreplaceable shortage of quinine just when their armies were moving deeper into malarious regions. Here was a medical emergency of grave proportions and immediate steps were taken to meet it by increasing atabrine production. It was necessary first to learn how to make the German intermediates which had been used in the manufacture of American atabrine. Thanks to the genius of American pharmaceutical chemists, and to coordinated activities of the National Research Council and the War Production Board, all difficulties were surmounted and by the end of 1943 military supplies were in reasonable abundance.

The great stimulus of war needs, and the fear that American atabrine was not equivalent to the prewar German product, led directly to an enormous amount of research into the chemistry, pharmacology, toxicology, and clinical effects of this drug. The National Research Council assigned many workers to the problem and it was not only proved that American and German atabrine are identical but there was obtained in a single year far more information about the action of atabrine than had been published in the previous decade.

For instance, Shannon (12) and others disclosed that large initial doses of atabrine are required in order to maintain from the outset of treatment a plasma concentration adequate for antimalaria effect while at the same time meeting a relatively high tissue demand for the drug. This observation resulted in a revision of standard therapy and the present regime has proved to be considerably more satisfactory than the old one.

Plasmochin was little used by the Army but it has been included in the vast program for investigating antimalarial drugs. Several studies towards the end of the war confirmed earlier British observations that a combination of quinine and plasmochin, given over a period of 10 days, will result in the radical cure of a higher percentage of vivax patients than will any other type of therapy. However, plasmochin toxicity is still a limiting factor to be carefully considered.

Great as was the output of atabrine it did not suffice for both military and civilian needs. This deficiency led to renewed interest in totaquine, a standard-

ized mixture of cinchona alkaloids which had been recommended by the Malaria Commission of the League of Nations in 1931 (5) and which was on record as an effective substitute for quinine sulfate. It was possible to increase production of totaquine, using cinchona bark from Central and South America. By the work of several governmental agencies, and thanks in large measure to Colonel A. F. Fisher, who in 1942, brought Mindanao seeds through the Japanese lines, and grew them to the seedling stage in the United States, considerable progress has been made in developing cinchona cultivation in the Americas. At the present time fairly large supplies of totaquine are available. Although relatively little use is being made of this mixture it is cheaper than quinine and could have considerable value in areas where economic levels are low and especially where self-medication is the rule.

Relapsing vivax malaria should not have been a surprise. It has long been notorious and the experiences in Macedonia in World War I taught a painful lesson which, unfortunately, was forgotten. Southwest Pacific vivax cases, and the experiments of Coatney and others with a temperate zone strain, have again emphasized the difficulty in obtaining a radical cure of infections due to this species of plasmodium. The troublesome relapsing tendency in vivax infections, added to the quinine shortage, led to an intensive search for new antimalarials. By 1 July 1945, more than 12,000 drugs had been tested in this country as regards possible usefulness in malaria therapy. While no phenomenal drug has been discovered, yet certain forward steps have been taken. There are new antimalarials, not yet in production, which possess all the advantages of atabrine without the toxicity or the skin-tinting disadvantages. Moreover, one of these new antimalarials may be given in single weekly doses for suppressive treatment. So, the war-stimulated research in the field of malaria therapy has laid a substantial foundation for future work. When published, it will materially shorten the road for all who follow.

#### PROPHYLAXIS

During the early months of the war, in fact until the middle of 1943, the incidence of malaria in Allied Forces overseas was extremely high in certain areas. Once again, as in Macedonia, Palestine, and East Africa in World War I, and in many previous campaigns, it was clearly demonstrated that uncontrolled malaria may be a serious military problem.

It is not surprising, therefore, that the most outstanding advances in malariology in World War II were in the field of prophylaxis. These lessons included the effectiveness of atabrine suppressive treatment, the value of repellents, the improved use of airplanes for distributing Paris green, the practical importance of pyrethrum spray-killing, the unusual powers of DDT, and finally the most important lesson that malaria, where it is more than mildly endemic, can not be successfully subdued without a specialist control organization.

#### SUPPRESSIVE TREATMENT

Clinical prophylaxis or suppressive treatment of malaria was attempted even before causation of the disease was known. But results were never satisfactory.

For example, reports about prophylactic quinine in Macedonia in 1915-1917 were contradictory (6), and this was the history of all civil or military attempts at mass prophylaxis with quinine or atabrine before the war.

Because of conflicting observations comparatively little use was made of suppressive drugs during the first years of the war. But the serious loss in manpower due to malaria in 1942 led to a careful re-examination of the subject. By 1944, on the basis of studies fostered by the Armed Services and the National Research Council, there had been established a suppressive regime for atabrine at the rate of a tablet (0.1 gram) a day. This dosage was found to maintain the minimum plasma level required for clinical prophylaxis.

But wide differences of opinion still persisted as to the effectiveness of the drug in the field. The question was finally answered by the experiments of Brigadier Fairley (5). With the help of over 500 volunteers from the Australian Army, Fairley proved the certainty with which all clinical malaria can be suppressed, and falciparum malaria cured, by daily doses of 0.1 gram of atabrine taken for two weeks before infection, and for a month thereafter. From 10 to 300 infective mosquitoes, some with *P. vivax* and some with *P. falciparum*, were allowed to feed on each individual exposed in Fairley's series yet *not a single clinical break-through occurred* during the period of atabrine administration. In order to intensify the test, some men were subjected for an hour to a temperature of 0° F., others were marched 85 miles in three days, others were given insulin until the blood sugar dropped to 40 mgm., others had repeated adrenalin injections, some were exposed to 18,000 feet altitude change without increased oxygen, and up to 35,000 feet with increased oxygen, yet in no case did the atabrine fail to suppress all clinical symptoms and in no case did parasites appear in thick blood films while atabrine at the rate of 0.1 gram per day was being taken.

Although parasitemia was not patent, yet both *vivax* and *falciparum* cases were infective to non-immunes by blood inoculation between the 7th and 11th days after infection, proving that atabrine will not prevent infection. In the case of *P. vivax*, the blood remained infectious, and invariably there were relapses in due time after discontinuing the drug. But blood from those who had been initially infected with *P. falciparum* failed to infect non-immunes at the conclusion of the six weeks of atabrine suppressive therapy and the disease never became clinically manifest.

The tremendous advantages of this regime of suppressive atabrine are obvious:—no clinical malaria and no subsequent falciparum relapses. When a break-through occurs during treatment it is practically certain that atabrine has not been taken in the prescribed manner.

Here is one of the outstanding advances of the war. Disciplined and trained soldiers or civilians under this regime of atabrine prophylaxis can now carry out their missions unhampered by clinical malaria, no matter how high the local endemic level may be.

#### REPELLENTS

Out of research stimulated by the war have come some mosquito repellents of considerable value. Those which were most used during the war were Rutgers

612 and, to a greater extent, dimethylphthalate. These synthetic liquids will give complete protection against mosquitoes for 90 minutes under experimentally controlled sweating conditions. In the field they may protect for as long as four hours after liberal application.

Toward the end of the war better methods for evaluating repellents were developed, such as the psychrometric chamber in which it is possible to standardize observations for various types of climate. By the use of this device, certain mixtures have been found to give complete protection against mosquitoes for periods up to 300 minutes under sweating conditions.

#### PARIS GREEN

Paris green had been distributed from airplanes on a limited scale prior to the war. Seldom did loads exceed 700 pounds of diluted larvicide. But military needs stimulated experiments which in the Mediterranean Theater led to use of planes carrying 3000 pounds of mixture at a time. Excellent malaria control results over wide areas were achieved. Some idea of the magnitude of dusting in Corsica, for instance, may be surmised from the fact that one pilot alone distributed more than half a million pounds of Paris green mixture during the 1944 season.

#### SPRAY KILLING

Although household pyrethrum sprays came into use in the United States in 1919, their importance in malaria control was not realized until 1935 when Thornton (13), published observations from South Africa. These reports led to field studies in India by Covell (3) and others (11) which clearly indicated the great practical value of such sprays in malaria prophylaxis.

A notable improvement in technique of applying pyrethrum sprays came when Goodhue and Sullivan (7) in 1942 reported that pyrethrins can be effectively dispersed, for adult mosquito spray-killing, from pressure cylinders containing liquid freon-12. As the freon-insecticide mixture is sprayed it forms a fine mist called an aerosol. Eighteen ounces of freon-pyrethrum mixture are sufficient to spray effectively 150,000 cubic feet of space.

The Army became actively interested in spray-killing as a malaria control measure in 1942 and fostered experiments to make it more practical. During the summer of that year engineers of the Westinghouse Company and Doctor Goodhue of the Bureau of Entomology and Plant Quarantine cooperated with the Sanitation and the Tropical Disease Control Sections of the Surgeon General's Office, in devising, and testing in the field, the first practical freon-pyrethrum "bomb". The Armed Forces used millions of such cylinders during 1943, and subsequently, and there is no question that they represent a most important advance in malariology during World War II.

#### DDT

Vastly stimulated by military needs, a substance, first synthesized in 1874, has become available as an insecticide of great antimalaria value in that it not



only has larvicidal properties but also a prolonged residual effect against adult mosquitoes when sprayed over the surfaces upon which they commonly alight or spend their daytime resting hours. This substance is the much publicized DDT or dichloro-diphenyl-trichloro-ethane (15).

Certain insecticidal properties of DDT were discovered in Switzerland in 1939. In 1942, a sample was sent to the United States Department of Agriculture. Since that time the Department has been testing DDT at its Orlando branch and has found it to be a highly effective insecticide. Exceptional credit is due the Orlando group whose laboratory, under the able direction of Doctor Knipling, became the world's focal point for basic DDT studies. These workers were first to discover the practical value of DDT in malaria control. The results of Orlando tests, and of studies by numerous Allied Army, Navy, and civilian units all over the world, have led to a large production program.

Undoubtedly, DDT is of great value in residual mosquito spray-killing. When a suitable solution is applied to an average resting place, the DDT will kill mosquitoes (and flies) alighting on it, and this effect will be apparent for three months or longer. Only a few minutes of contact are required, with either airborne or surface particles, after which the insect will die in from four to six hours.

DDT solutions sprayed as an aerosol or fog will kill adult mosquitoes out-of-doors. This so-called *barrier treatment* may be effective for a week or more depending on dosage, rainfall, and perhaps other factors. A great deal of experimental work has been done by the Army, Navy, and Public Health Service, and by the British, in developing airplane spraying and fogging with DDT. Entire beachheads have been made mosquito-free in this way. But the effect on other insect life, on pollination by insects, and on various biological balances is not well known and may be an important drawback to such area control.

One regrets that the word "magic" has so often been used to describe this new weapon. DDT is a sharp tool which must be employed intelligently. Probably the greatest use of this insecticide in civilian malaria control will be in careful application to individual buildings and other sheltering places of malaria vectors, and to certain larva breeding places. But no one who has worked with DDT questions the fact that it represents a very real advance towards economical control, especially in tropical areas.

#### ORGANIZATION

One of the basic lessons which had to be re-learned in World War II is the fact that a standard medical and sanitary table of organization does not meet the needs of a malarious theater of war. Routine camp cleanliness, good food, physical fitness, adequate hospital facilities, none of these, nor all combined, will prevent or control malaria. Moreover, one can not hope to achieve results merely by supplying quantities of supplies, such as atabrine and DDT. Over and over again, in civil life and military, it has been proved that malaria can not be successfully controlled, in any but lightly endemic areas, or in small groups of individuals, without a professional malaria control organization to survey, plan, execute, supervise, and maintain the numerous technical procedures continuously required (10, 14).

Malaria control is possible anywhere in the world, but success will depend on men trained and organized, and backed by administrative authority. Under military and under civil conditions, effective prophylaxis is difficult or impossible without a malaria-conscious high command. Moreover, experience has shown that a civil law, or military directive, to be effective must be supplemented by persuasion or salesmanship which is based on skillful instruction and propaganda designed to interpret the philosophy behind seemingly arbitrary rules. Adequate implementation of this, and the other components of a malaria control program, requires the undivided attention of a specially trained malaria control organization of a size commensurate with the problem.

In the early months of World War II it was again demonstrated, as in Macedonia in the First World War, that the day of amateur malariologists has passed. The belief epitomized by the statement that "a doc is a doc" has not been valid for a long time and it had painful results in practice in the early months of the war. Troops were overwhelmed with malaria in several areas. Not until a special malaria control organization came into effective operation overseas, by the middle of 1943, did rates begin to fall. As a direct result of the work of this specialist organization, monthly hospital admission malaria rates fell from one, two, and even three thousand or more per thousand per annum to 50, 20, and even 10 per 1000 per annum in some of the world's most malarious areas. Better and more abundant antimosquito weapons, and suppressive atabrine, were useful but only because there was a specialist organization to make them effective. Contrary to press statements, it definitely was not DDT which brought down the rates. They subsided more than a year before DDT became available in practical amounts. The decisive factor, as stated in a recent Army Medical Bulletin, was the work of the special medical malaria control organization (2).

It seems fitting to pay special tribute to the skill, courage, and devotion to duty, of the malaria control organizations of the Army and of the Navy by quoting the following message which Brigadier General Guy Denit (4), Surgeon of the Southwest Pacific Theater, published at his headquarters in December, 1944.

"The reduction of the malaria attack rate in this theater to a point at which it no longer contributes a dangerous handicap to our military effort is an achievement of historical importance in preventive medicine. It has been the result of a joint effort which is to the great credit of all who have participated. In this accomplishment the malariologists and the malaria survey and malaria control units have played the major rôle. Despite hardships and often danger, their achievements have been notable. The Medical Department is proud of your initiative and perseverance, of your professional contributions, and of the striking success of your efforts."

So here was the most important lesson in malariology from World War II, teaching once again with renewed emphasis the simple but exceedingly important fact that malaria can be controlled anywhere in the world in any environment, no matter how chaotic, when trained and organized personnel are given the necessary supplies and authority. Costs under military conditions are always huge. But it has already been proved that under civil conditions malaria trans-

mission can be completely stopped at a price within the economic reach of many rural communities even in the tropics (11).

The greatest social obstacle to malaria prophylaxis in the world today is the lack of suitable malaria control organizations. Economic barriers have been removed by spray-killing. Rural malaria control in many areas can now be one of the least expensive of public health measures. But until there is wide acceptance of the outstanding lesson about essential organization, so clearly demonstrated again in World War II, malaria will remain a paramount disease.

Allied malaria control units have demonstrated the value of malaria control by modern methods all over the world with such striking success that civilian authorities are more willing than ever before to budget funds for antimalaria programs. Already there are plans in hand for extensive work in such widely separated areas as the southern United States, Brazil, West Africa, Italy, India, and Australia, in each case based to a considerable degree on lessons from World War II.

One may reasonably hope that, with suitable organization, malaria will be eradicated from the United States within the next decade, and that in many tropical areas, even though economically depressed, this disease, now of the greatest importance, may become in the next half century one of the least of public health problems.

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# EPIDEMIOLOGY AND INCUBATION PERIOD OF JAUNDICE FOLLOWING YELLOW FEVER VACCINATION<sup>1</sup>

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In 1883 Bremen had an epidemic of jaundice following mass vaccination with human vaccinia lymph (1). More recently several outbreaks of jaundice have been described following the use of vaccines and serums (2-5). In each instance human material was a component of the suspected vaccine or serum. During 1942 a considerable epidemic struck American troops in this country and abroad (6). This paper contains an epidemiological study of a part of this outbreak. The epidemic was preceded by mass inoculation of troops with yellow fever vaccine (YFV) prepared by suspending the 17D strain of virus in presumably normal human serum. The virus was grown in chick embryos and the serum was used as a virus preservative. Observations have been made that epidemic jaundice may be precipitated by parenteral use of other biological products like insulin (7) and arsenicals (8). Certain investigators are of the opinion that some epidemics, including the one under discussion, probably were caused by an infectious agent unwittingly carried in the serum of donors used as sources of material (2, 4-6).

In this paper the term "jaundice" is meant to include sporadic cases commonly called "catarrhal jaundice", and epidemic cases of the same or a similar disease, whether or not associated with previous injections. It is not intended to imply that all similar cases of jaundice have a common etiology. The epidemiological relationship among sporadic, epidemic and post-immunization jaundice does not enjoy unanimity of opinion. However, there appears to be general agreement that there is a striking similarity among cases of jaundice occurring in all three circumstances and that they cannot be differentiated on clinical or pathological grounds (4, 5).

There are two cogent reasons for looking upon certain vaccines and serums as being responsible for epidemics of jaundice that appeared in their wakes. One is the high incidence of jaundice associated with particular batches of serum or vaccine and not with others prepared essentially in the same way. This suggests that some batches may contain an etiological agent of disease that is not present in the others. The other reason is the relative uniformity of the interval between inoculations and the appearance of jaundice. Some idea of this can be gained from table 1.

Although some uniformity in time can be seen in table 1, the range of time over which cases may occur following inoculation may be quite extensive. Also, it

<sup>1</sup> Forwarded to the Surgeon General, U. S. Army on September 20, 1943 requesting publication.

<sup>2</sup> Major, Medical Corps, A.U.S. Now, Dep't. of Medicine, Univ. of Chicago.

has been noted that the interval between inoculation and appearance of jaundice may vary in the same epidemic, depending presumably upon the lot of inoculum used. In Brazil, for instance, one lot had an "incubation period" of 17.8 weeks and another of 20.4 weeks (4). Similar variations were noted in the American Army epidemic of 1942 (6).

#### EPIDEMIOLOGY AT CAMP "BAKER"<sup>3</sup>

An epidemiological study of jaundice following yellow fever vaccine (YFV) was undertaken at Camp "Baker" in southeastern United States. It was possible to study the epidemic at this post from its beginning. Cooperation on the part of the Post Surgeon and members of his staff was excellent. A thorough search was made during the early period for all suspicious and clinically manifest cases of jaundice. Dispensary medical officers sought cases at sick call. Barracks were searched for indisposed soldiers who may have been ordered to quar-

TABLE 1

*Approximate intervals between immunization inoculations and appearance of jaundice*

EPIDEMIC	INOCULUM USED	APPEARANCE OF JAUNDICE AFTER INOCULATION
		<i>mos.</i>
Bremen (1) (1883-84) .....	Human vaccinia lymph	1.0- 7.0
England (2) (1937-38) .....	Yellow fever vaccine	1.2- 7.5
Brazil (4) (1939-40) .....	Yellow fever vaccine	0.5-18.0
Russia (5) (1939) .....	Sandfly fever vaccine	2.0- 4.9
American army (6) (1942) .....	Yellow fever vaccine	1.3- 6.0+

ters. Eyes and skin of men on mess lines were examined for jaundice as well as those that preferred to remain in quarters during meals due to loss of appetite. Suspicious cases were sent to the station hospital laboratory for icterus index determinations and clinical observations. Besides the appearance of jaundice and gastro-intestinal symptoms, muscular aching and fever also were considered as evidence of possible impending jaundice. It is unlikely that an important number of clinical cases was missed during the first half of the epidemic. There must have been, nevertheless, a considerable number of subclinical or abortive

<sup>3</sup> Key to military posts and YFV lot numbers:

Camp "Baker" is Fort Belvoir, Virginia.

Camp "Sugar" is Fort Sill, Oklahoma.

Camp "Love" is Fort Lewis, Washington.

"B1" is YFV lot 329

"B2" is YFV lot 364

"B3" is YFV lot 350

"B4" is YFV lot 351

"B5" is YFV lot 284

"M1" is YFV lot 368

"M2" is YFV lot 369

cases that never came under observation. The station hospital acted as the clearing house for the epidemic; all cases occurring during the rise of the epidemic were examined and questioned there. During the latter part of the epidemic many mild cases were allowed to remain ambulatory because hospital facilities were overloaded.

As each case appeared, he was questioned thoroughly in regard to his itinerary immediately prior to and since his association with the military service. Changes in posts, units, and barracks were recorded with dates of such changes. Types and places of duty were considered. Immunization records were studied for the date of yellow fever vaccination and the lot of YFV received. Information obtained from individuals was cross-checked with units, dispensary records and unit personnel so that accuracy of records could be evaluated. A large engineers' map which showed post facilities in detail proved to be of considerable value when used as a "spot" map. An appropriate pin was placed in the figure representing the quarters of each case as he appeared in turn.

The population of Camp "Baker" was fairly stationary in number, ranging approximately between 18,000 and 19,000 from April 1941 to August 1942. Approximately half the post consisted of relatively transient basic training and officer training units. The former remained on the post between 8 and 13 weeks but groups moved in and out on a staggered weekly schedule. Officer candidates remained on the post for periods of 12 weeks. The permanent half of the post population could be studied to better advantage because of the availability of records and detailed histories of movements. Nevertheless, the entire population was studied.

The epidemic of jaundice at Camp "Baker" coincided in time and duration with the large epidemic among American troops during 1942. Figure 1 demonstrates the distribution of all cases hospitalized by weeks and the general relationship to administration of YFV. Vaccine was given at two periods. A group of about 500 men received YFV on December 30, 1941 and during the first week in January 1942. The remainder of the troops were vaccinated between February 23 and March 9, 1942, except for stragglers. The plain and stippled areas in the background of figure 1 show the relative proportions of vaccinated to unvaccinated population at all times. Therefore, the unvaccinated cases between May and December, 1942, represent a considerable increase in attack rate for the unvaccinated portion remaining. Table 2 shows stationary units that were studied and the lot numbers of YFV they received. These units plus basic training, officer candidate and enlisted men's schools made up the bulk of the post population. Student troops came from various posts and many had received YFV from a variety of lots at different times. Detailed data in regard to YFV could not be considered accurate for these people.

YFV lot "B2" was used also on the post. Relatively few cases of jaundice had received this lot. The number was uncertain and extremely small. No record of the number of doses of lot "B2" was discovered. If other lots were used at Camp "Baker", no such evidence was available and no cases of jaundice were found to be associated with them. A number of individuals suspected of not

having received YFV became jaundiced also. Blood specimens from six such individuals were tested for neutralizing antibodies against yellow fever virus by the Laboratories of the International Health Division of the Rockefeller Foundation in order to learn whether or not they had received YFV. Antibodies could not be demonstrated in any case. These six cases constituted a random sampling of an estimated 20 to 30 unvaccinated cases. The sample comprised all unvaccinated cases that had not yet left the post at the time of sampling. Fortunately, this group included a distribution of cases covering the entire epidemic period. Figure 1 shows the exact distribution of the first three un-

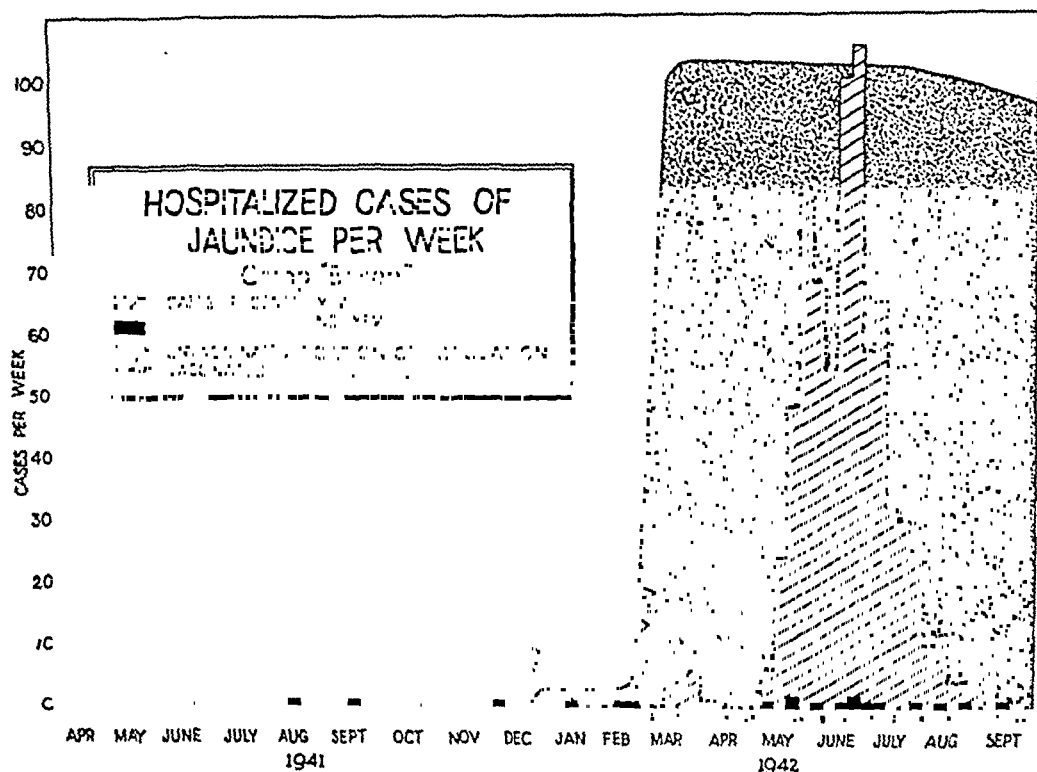


FIG. 1. OCCURRENCE OF HOSPITALIZED CASES OF JAUNDICE AGAINST A BACKGROUND OF VACCINATED AND UNVACCINATED PORTIONS OF THE POST POPULATION

The entire vertical axis of the graph box represents 100 per cent

vaccinated cases in May 1942 and the approximate arrangement of the other unvaccinated cases during the epidemic period. Although 20 to 30 cases apparently had not been vaccinated according to records, an arbitrary figure of 12 cases (50 per cent of the estimated number) will be used in calculating rates in order to allow for possible error in sampling. One early unvaccinated case was a civilian that worked on the post. He was hospitalized May 11 and died within a few days. The post mortem findings revealed hepatitis indistinguishable from that seen in vaccinated cases.<sup>4</sup> His blood and ascitic fluid were tested for yellow fever neutralizing antibodies also and none were found.

<sup>4</sup> Post mortem was performed by Lt. Col. Balduin Lucké of the Army Institute of Pathology.



It is expedient at this point to establish various attack rates for the purposes of this study. These appear in table 3. Altogether, slightly more than 700 cases or approximately 3.8 per cent of the total post population was hospitalized with jaundice. The peak over-all incidence for one barrack was about 25 per cent.

TABLE 2  
*Stationary units and YFV lots received by them*

UNIT	YFV LOT	VACCINATION PERIOD
"XA" Company.....	"B1"	Dec. 1941-Jan. 1942
"Y" Battalion.....	"B1"	
"Z" Regiment.....	"M1"	Feb.-Mar. 1942
"V" Regiment.....	"M2"	
Post Detachments.....	"M2"	
"Q" Battalion (Company "C").....	"M2"	
"XB" Company.....	"M2"	
"XC" Company.....	"M2"	

TABLE 3  
*Basic attack rates of jaundice*

	ATTACK RATE PER 1000 PER WEEK
Preepidemic* (army during Jan. & Feb. 1942).....	0.01
Preepidemic (Camp "Baker" population from April, 1941 to March, 1942).....	0.01
Epidemic period (Camp "Baker" population from March, 1942 to October, 1942).....	1.40
Epidemic period† (unvaccinated individuals).....	0.36
Peak week‡ (Camp "Baker's" early group, March 24-30, 1942)....	8.00
Peak week (Camp "Baker" population, June 23-29, 1942).....	5.50

\* Rate obtained from official report of 125 cases per month during January and February, 1942, for the Army within the continental limits of the United States and total strength for that period as reported by the War Department in the public press.

† Unvaccinated military personnel estimated at about 1200 or 6.5 per cent during the epidemic period.

‡ This group received YFV lot "B1" which is considered to be relatively "non-icterogenic". It was not associated with a high incidence of jaundice in the total military population.

#### *Period prior to main epidemic*

The first six cases of jaundice admitted to the station hospital since its establishment in April 1941, had not received YFV. They occurred sporadically during a period of seven months as shown in figure 1. A battalion ("Y") consisting of two companies and a separate company ("XA") were inoculated with YFV lot "B1" on December 30, 1941 and during the first week of January in

anticipation of early departure from the post. Nine cases of jaundice occurred in this group of approximately 500 soldiers. The arrangement in quarters and the distribution in time with relationship to the date of vaccination is shown in figure 2.

Figures 2-5 contain two parallel, vertical lines. In each figure these lines embrace a diagram showing the distribution of cases in barracks. The cases shown in barracks are arranged below, between the parallel lines, according to appearance in time also. Except for figure 2, the period enclosed between the verticals represents the first wave of the main epidemic. The peak of this wave occurred the week of May 26-June 1.

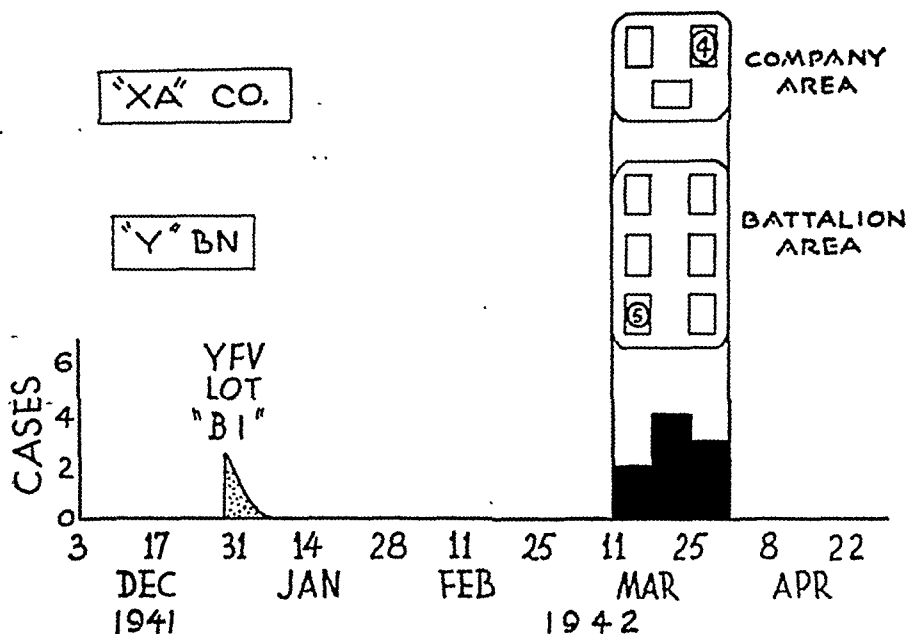


FIG. 2. OCCURRENCE OF CASES IN BARRACKS AND TIME AND RELATIONSHIP TO YFV INOCULATION

Each barrack in figure 2 quartered approximately 50 men. This was the usual barrack population except as noted later in an area of brick barracks. All four cases in "XA" Company occurred in one of three barracks. The other five cases were quartered in one of six barracks of "Y" Battalion. The probability of such distribution by chance is 1 to 80 for "XA" Company and 1 to 7,700 for "Y" Battalion.

#### *Main epidemic*

The bulk of the post population, except for stragglers, was vaccinated between February 23 and March 9, 1942. The main epidemic was continuous from May to September as shown in figure 1. The incidence of disease rose rapidly during the first four weeks and reached a peak the last week in May. The incidence declined progressively for two weeks and rose sharply again to a maximum of 105 cases during the last week of June. Subsequently, the epidemic fell off so that the incidence almost had regained the preepidemic level by September.

Units that were involved in the epidemic wave starting in May will be considered now. The distribution of cases in barracks and in time during the first major epidemic month for individual organizations is demonstrated in figures 3-5. "Z" Regiment is represented in figure 3. The three barracks affected first belonged to Company "A". The first case in the regiment occurred in Company "A" in December, 1941, prior to issue of YFV. Then 11 cases occurred consecutively in the same company during May and two more in June before a case occurred in any of the other three companies. The probability of 13 consecutive cases occurring in one of four companies by chance is 1 in 67,000,000. All members of the regiment received either YFV lot "M1" or "M2" during the

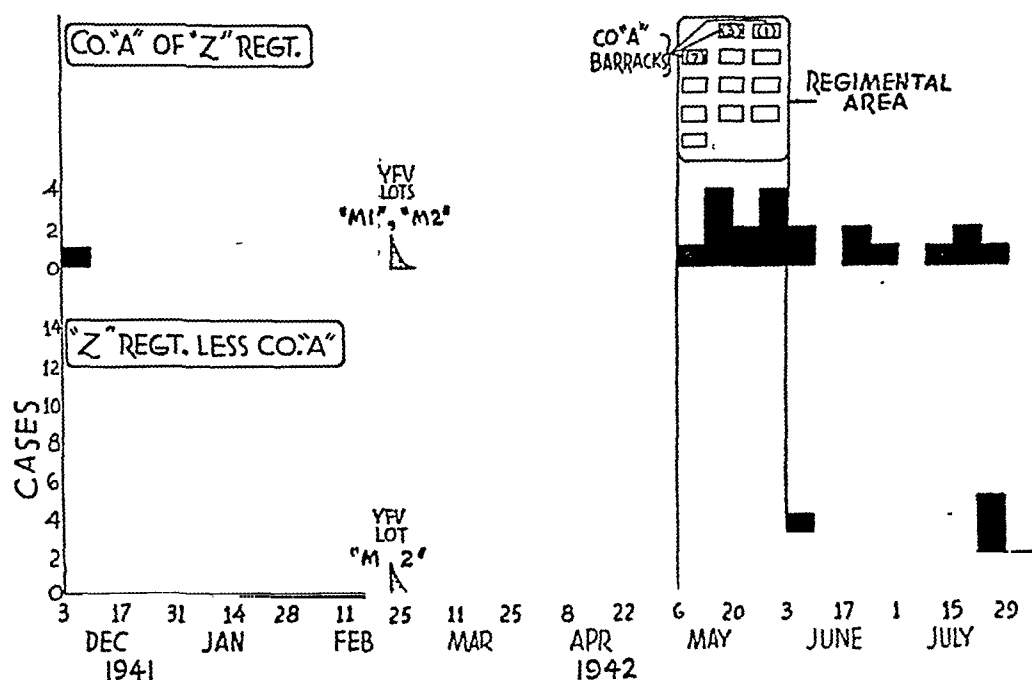


FIG. 3. SAME AS FIG. 2

first week in March. Temporal grouping also is seen in this regiment. "M1" and "M2" are both considered to be highly and equally "icterogenic".

A promising experiment appeared fortuitously in "Z" Regiment when 400 recruits were mixed thoroughly with about 500 seasoned troops on June 1, 1942. The latter group had received YFV and were having considerable jaundice while the fresh troops had not received vaccine and had no jaundice. Since the attack rate for unvaccinated individuals during the epidemic period was 0.36 per 1000 per week, the expectancy for jaundice in the group of 400 was about 1 case for the period of the epidemic that remained following an incubation period of 3.2 weeks. This period will be shown to be significant. No clinical cases were observed among the 400 recruits but the unvaccinated wife of one soldier from this regiment became jaundiced. Unfortunately, the size of the test group was not large enough to be a source of positive information.

One area consisted of large brick barracks that quartered about 200 men each. Figure 4 shows the barracks that housed men all of whom received YFV lot

"M2". Men in other barracks of this area did not receive the same vaccine. Building 201 contained the post detachments. Buildings 213 and 203 represented the Headquarters and Service Companies, respectively, of "V" Regiment. The Service Company was composed of Negro personnel, whereas the other three barracks under consideration contained only white troops. Building 200 was the barrack for Company "C" of "Q" Battalion.

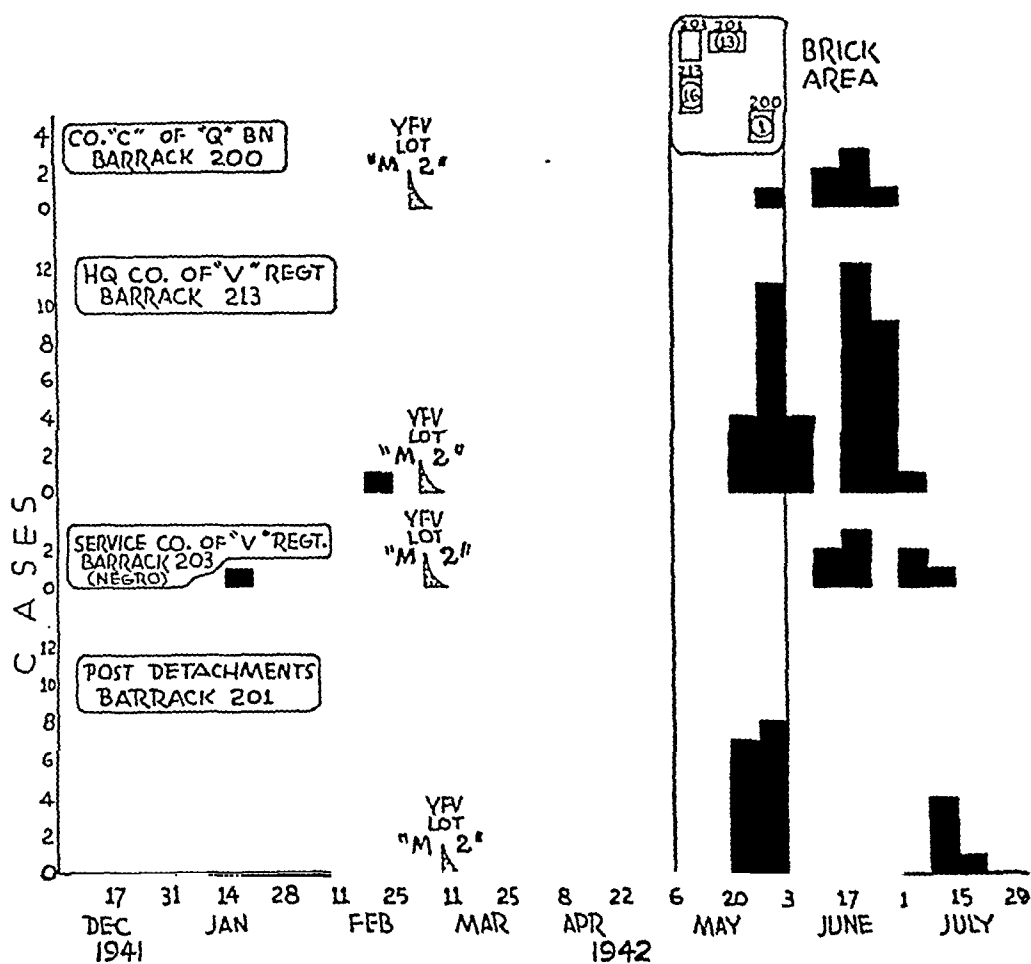


FIG. 4. SAME AS FIG. 2

Vaccination was carried out during the period March 2-5 for all except the post detachments. These units were inoculated March 9. The probabilities that buildings 213 and 201 would have 7 and 8 cases, respectively, before any appeared in building 200 are 1 to 120 and 1 to 250 by chance. The probabilities that the same two barracks would have 20 and 13 cases before any occurred in 203 are 1 to 1,000,000 and 1 to 8,000 respectively. Temporal spacing of groups in these barracks is striking. It is interesting to note the preepidemic appearance of jaundice in buildings 213 and 203.

Two separate companies, "XB" and "XC", were inoculated with YFV lot "M2" on March 3 and 2, respectively (see fig. 5). The probability that 9 out

of 10 cases would fall in 1 of 2 companies by chance during the first month is 1 in 100 times.

The relatively transient population is represented in figure 6 by the officer candidates and the basic training troops. The temporary status of these people

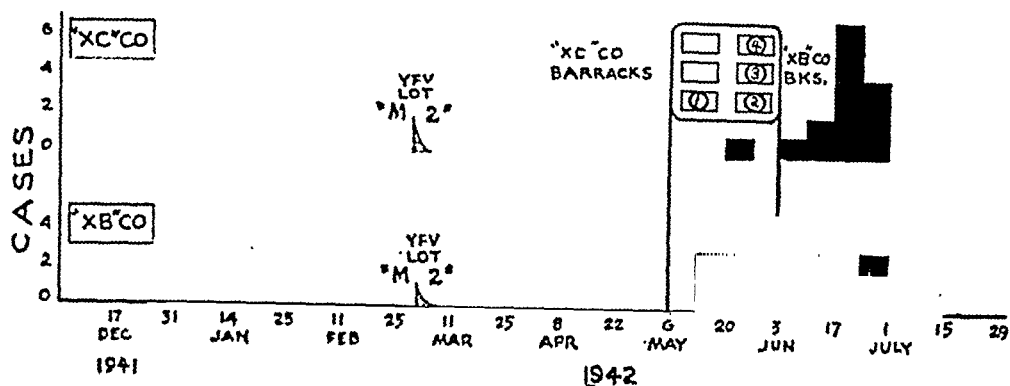


FIG. 5. SAME AS FIG. 2

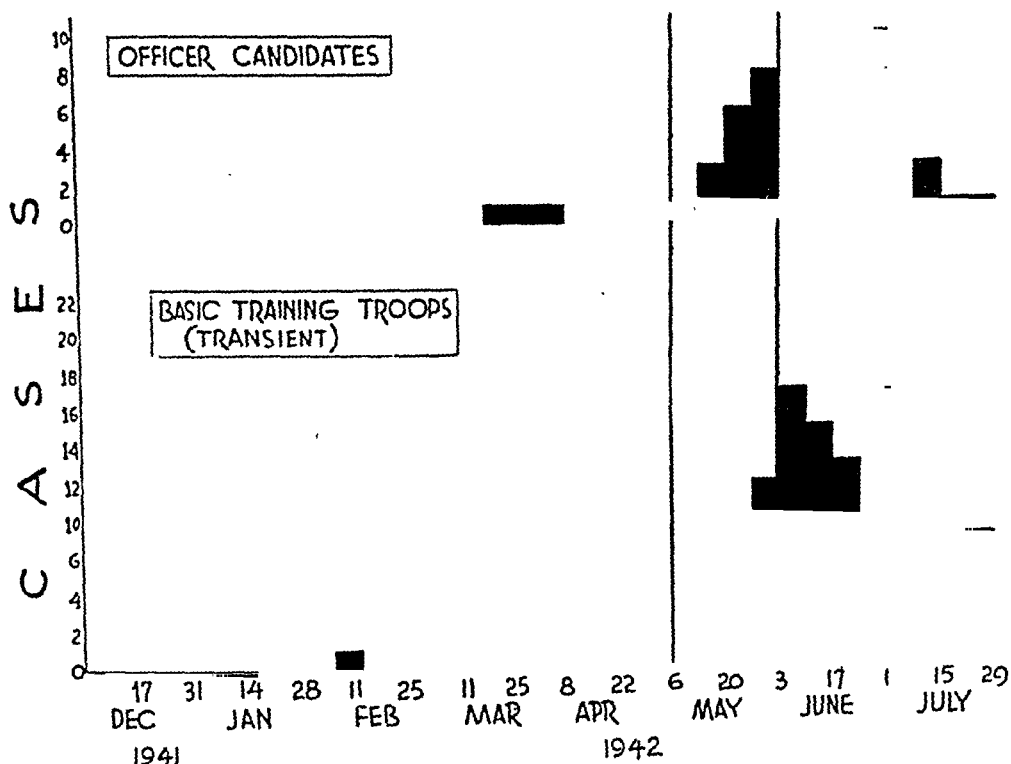


FIG. 6. OCCURRENCE OF CASES AMONG OFFICER CANDIDATES AND BASIC TRAINING TROOPS IN RELATIONSHIP TO TIME

made it difficult to obtain adequate data about them in regard to YFV administration. Cases in these units were scattered among many barracks during the early part of the epidemic but later began to occur in clumps. Temporal spacing of groups is noted also among these school troops.

## INCUBATION PERIOD

Figures 2-5 contain two different regularities in time. One is the interval of time between vaccination and the onset of epidemic jaundice. Table 4 lists these intervals for the units under consideration. The average is 12.3 weeks.

TABLE 4  
*Time of onset of jaundice after YFV administration*

UNIT	ONSET WEEKS AFTER YFV
"Y" Battalion ("XA" Company).....	11
"Z" Regiment (Company "A").....	11
"Z" Regiment (less Company "A").....	15
"Q" Battalion (Company "C").....	13
"V" Regiment (Headquarters Company).....	12
"V" Regiment (Service Company).....	15
Post Detachments.....	11
"XC" Company.....	12
"XB" Company.....	11
Average (mean).....	12.3

TABLE 5  
*Calculated mean, standard deviation and coefficient of variation of intervals between peak incidences of cases in units*

	WEEKS IN DURATION		
	First peak interval	Second peak interval	Third peak interval
"Z" Regiment (Company "A").....	2	3	4
"Z" Regiment (less Company "A").....	3	4	
"Q" Battalion (Company "C").....	3		
"V" Regiment (Headquarters Company).....	3		
"V" Regiment (Service Company).....	2		
Post Detachments.....	4	2	
"XC" Company.....	4		
"XB" Company.....	3		
Officer Candidates.....	3	3	
Basic Training Troops.....	4		

Difference in onsets between Company "A" and other companies in "Z" Regiment.....	4
Difference in onsets between Headquarters and Service companies of "V" Regiment.....	3
Average interval (mean).....	3.2
Standard deviation or coefficient of variation.....	$\pm 0.72$ or 22.5%

Jaundice occurred in epidemic proportion after a similar interval whether vaccine was given in December 1941 or the following February.

The other regularity is the temporal spacing among groups of cases within units and barracks. The total elapsed time during which new case incidence was followed in figures 3 to 6 was between 9 and 12 weeks. The intervals between peaks are seen readily in figures 4 and 6 to lie between 2 and 4 weeks. Also, the less striking groups in figures 3 and 5 fall at 2 to 4 week intervals. Table 5

lists all intervals between peaks including the interval between the appearance of jaundice in Company "A" of "Z" Regiment and the onset in the other three companies, and the interval between the onsets in the Headquarters and Service Companies of "V" Regiment. The average for all intervals is 3.2 weeks. All 17 intervals, therefore, are 2 to 4 weeks in duration. According to figure 7, the interval mode as well as the mean is 3.2 weeks. The standard deviation is  $\pm .072$ , and the coefficient of variation is 22.5 per cent. The clustering of all 17 peak intervals about the mode of 3.2 weeks strongly indicates that the interval is significant.

Figure 1 shows two major waves, one reaching a peak during the last week of May and the other during the second half of June. The simultaneous appearance of peaks at these two periods within units and barracks apparently accounted for

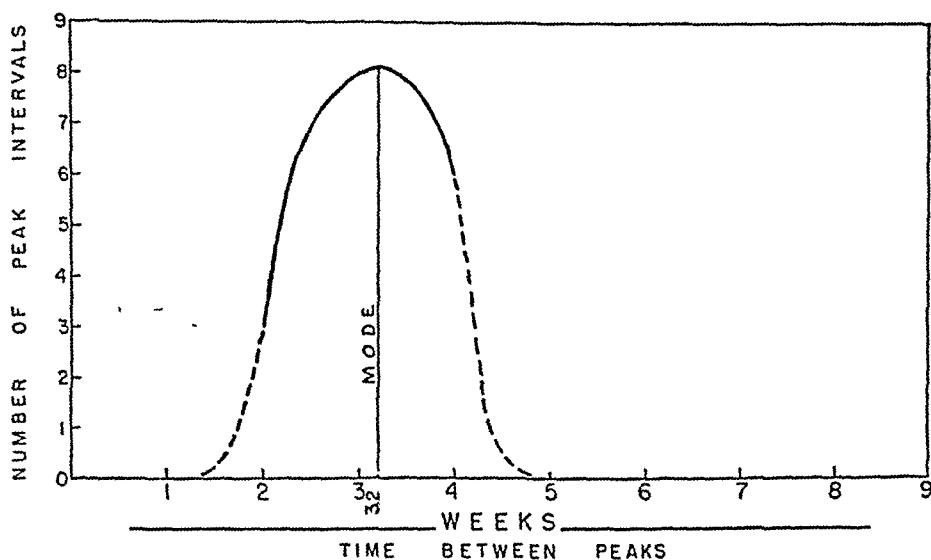


FIG. 7. MODAL CURVE FOR INTERVALS BETWEEN PEAKS OF CASE INCIDENCE AND INTERVALS BETWEEN ONSETS IN SIMILARLY VACCINATED BARRACKS

the major waves (see figs. 3-6). The 3.2 week interval observed between peaks is, therefore, a reflection of the mode shown in figure 7.

#### TRANSMISSION

The platoon was the smallest type of group apparent in this study. In garrison the entire platoon was usually housed in the conventional type of wooden cantonment barrack and consisted of approximately 50 to 60 men. Members of a platoon, consequently, had common sleeping and latrine facilities. Also, the platoon usually worked as a unit. Customarily, three platoons constituted a company. All members of a company messed together and had considerable contact among themselves. However, the tendency for contact among individuals of different companies was very much less. They were not likely to meet except during social hours. Even then "buddies" were frequently company mates. The selection by jaundice of individual barracks or platoons in several instances suggests that either close living or the common latrine, or both, were

important in the distribution of the disease. The company mess appears to have escaped indictment although it was not necessarily absolved under all circumstances. Mess under field conditions may not appear as innocent as it does in garrison. Although there appeared to be no natural insect vector involved, the fly as a mechanical vector was not exonerated. Although jaundice reached epidemic proportions in northern latitudes in March, prior to the issue of the year's fly crop, they were present in variable numbers at Camp "Baker" in several mess halls and in an occasional latrine. Recurrent failure of the flushing mechanism in one latrine gave rise to persistent fecal odor and the swarming of flies. This latrine was in a heavily affected barrack of Company "A" of "Z" Regiment. A prevaccine case of jaundice was quartered here. However, there were no confirmatory observations. Before the onset of the epidemic and during the early part, mosquitoes were either absent or present in small numbers. It is most unlikely that any one insect could have been a vector in the epidemic throughout the country in the early spring.

#### ACCESSORY DATA

In a large routine Army serological laboratory a decided rise in the incidence of jaundiced serum specimens was noted during May 1942. The laboratory officer was not aware of an epidemic of jaundice and considered the change to be related to antiluetic therapy. Investigation proved that half of the icteric specimens belonged to civilians that were having routine examinations either for induction into military service or for employment in civilian capacity. They gave no serological evidence of syphilis and, almost certainly, had not been given YFV.

Four wives of soldiers were hospitalized in an Army general hospital toward the end of September and beginning of October, 1942. The husbands of three had had jaundice and either had lived at home or had visited their wives frequently. The husband of the fourth was abroad but she mingled to a large extent with people in the military service. All four gave negative tests for neutralizing antibodies against yellow fever virus. Attention of this laboratory was called to these cases because it was exceptional in the experience of the admitting medical officer to see jaundice in his clinic.

#### JAUNDICE AT OTHER POSTS

It has been generally recognized that the use of certain lots of YFV was associated with large outbreaks of jaundice (6). However, at least two Army posts that took part in the major epidemic had many cases that received presumably "non-icterogenic" lots of YFV. Camp "Sugar" in south central United States had 172 cases of jaundice distributed according to figure 8 (9). Lots "B3" and "B4" do not belong to the "icterogenic" group of YFV lots, but were significantly involved in the epidemic at this post.

The same was true of YFV lot "B5" at Camp "Love" in northwestern United States (9). Lot "B1" was associated with the early group of cases at Camp



"Baker" and is considered to be relatively little "icterogenic" as an influence in the Army as a whole. Army units stationed in the Pacific area have reported epidemic jaundice occurring 9 to 13 months following the use of YFV (9). This is considerably longer than the usual post-vaccinal interval noted previously.

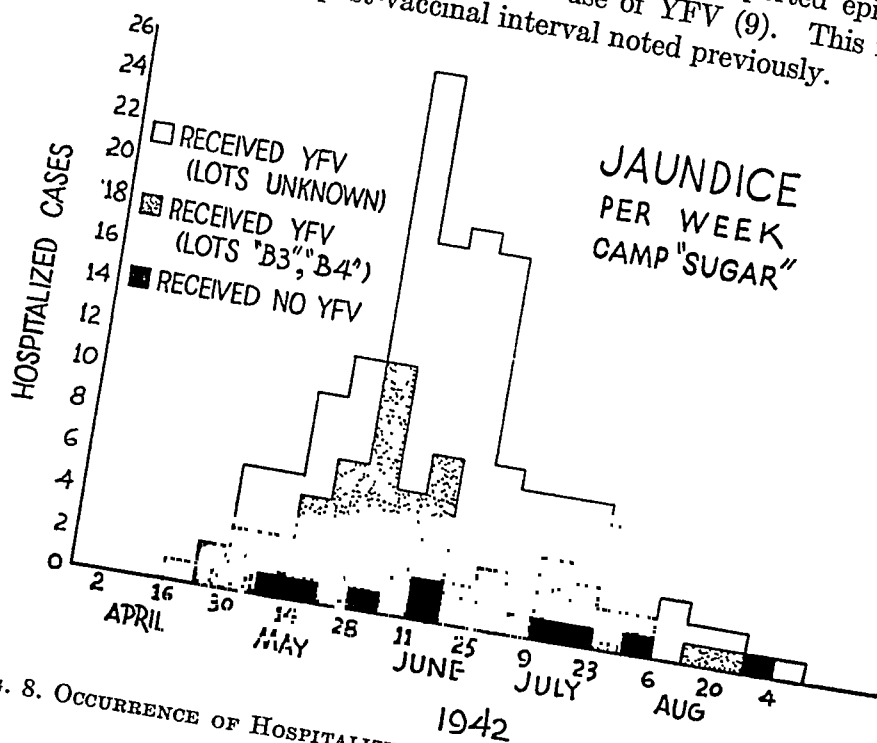


FIG. 8. OCCURRENCE OF HOSPITALIZED CASES OF JAUNDICE AT CAMP "SUGAR"

#### DISCUSSION

There appear to be two factors interwoven in this epidemiological pattern. One is YFV and the other is focal grouping of cases. Strikingly similar circumstances were observed previously in Brazil (4).

In regard to the focal distribution of cases encountered in Brazil, Fox, et al. stated: "With respect to lot 489, the contrast between the 0 per cent incidence in Morro de Argolas and the 19.19 per cent incidence in Melgaco is most striking. As for lot 494, although examples of abnormally high incidences are shown, the long list of localities with a 0 per cent incidence is of particular interest. On the basis of 1.56 per cent overall incidence for the lot, only one in 10 groups of about 200 vaccinated persons should fail to contain a case of icterus. Actually there were seven such instances in twenty-one groups of that approximate size."

In regard to incidence in households, they said: "This . . . includes individual probabilities calculated as low as 1:1,000,000, 1:5,000,000, 1:50,000,000 and even 1:1,000,000,000. It thus seems reasonably clear that the distribution of cases within households does not conform to that which would be expected if the

the occurrence of cases in the population vaccinated with lots 489 and 494 were governed only by chance."

Fox, et al concluded that the Brazilian epidemic most likely was caused by an agent residing within YFV and that the uneven distribution was based on a secondary factor. This factor they believed to be non-infectious and probably associated with dietary habits of certain families in certain affected communities. The hypothetical factor was not discovered. Also, the authors considered the disease to be different from the commonly seen epidemic jaundice on two accounts, although the diseases admittedly were indistinguishable on clinical and pathological grounds. They considered the attack of an adult group in their epidemic to be unique because epidemics that did not follow immunizations were thought to affect children, mainly. Also, they believed that there was a difference in incubation period.

These investigators apparently did not take into account the several epidemics that occurred in armies prior to 1938 that were not associated with vaccines or serums (6). It is noteworthy that a considerable epidemic attacked British troops in 1942 and they had not received suspicious inoculations (10). In regard to the "incubation period" in Brazil, it was assumed that the interval between the time of inoculation with YFV and the appearance of clinical disease was the incubation period. By such a priori reasoning the etiological agent was placed inadvertently but necessarily in the vaccine. Similar reasoning permeates epidemiological studies of jaundice when either vaccine or serum was involved (5-6). Even in experimental transmission studies little attention is given to the possibility of accidental cross-infection among experimental subjects (11). It is interesting to note that practically all cases in the Russian experience either were living or had lived in the same building (5). An outbreak of jaundice was observed by Soper and Smith (14) in Brazil following the use of YFV in conjunction with monkey antiserum. Human serum was not included at that time.

The Camp "Baker" epidemic contains significant grouping of cases both temporally and spatially. It would be stretching credulity to assume that an ingested toxic factor explained the grouping of cases in the American Army. Both the universal appearance of cases and the standard of dietary control throughout the Army cast doubt on the probability that an ubiquitous food substance accounted for the focal design of the epidemic in this country.

There is a considerable discrepancy in time between the 3.2 week incubation period determined by studying the distribution of cases during the epidemic and the period reckoned from the day of YFV inoculation. The latter period has a mode of either 12 or 16 weeks, depending on whether the whole epidemic period is considered to be either several waves or one (see fig. 1). All three intervals appear to be significant but only one can represent the true incubation period. If either the 12 or 16 week period is considered to be etiologically significant, the selective grouping of cases rather than chance appearance at the onset of the epidemic is difficult to understand. On the basis of a 12 week incubation period a hypothetical vaccine-born etiological agent

would be expected to effect the primary wave, and then a secondary wave by contagion would be expected to follow, in order to account for the prolongation of the epidemic period. The incubation period of the second or contagious wave would appear to have been curtailed from 12 to 3.2 weeks. Also, secondary cases would have had to have been recruited from unvaccinated individuals, those that were vaccinated with unincriminated vaccine, and those that did not prove to be susceptible to a hypothetical vaccine-borne dose. The secondary group would have had to have been considerably larger than the original susceptible group at Camp "Baker" according to the relative sizes of the primary and secondary waves. Also, the vaccine-borne dose must not have increased the immunity of the secondary group beyond the point of susceptibility to the contagious dose. Actually, the first wave included unvaccinated individuals and the second wave was made up largely of individuals that received "icterogenic" YFV. Also, the early group of cases in March had received vaccine of presumably low "icterogenicity".

On the basis of a non-contagious disease with a 16 week incubation period the grouping of cases in time and place also remains unexplained. Neither is a 36-fold increase in attack rate during the epidemic among the unvaccinated understandable on a non-contagious basis. If the disease is looked upon as communicable to secondary cases, an incubation period of 16 weeks would be expected to show an accumulation of secondary cases during September and October. In reality, the epidemic had largely depleted itself by September.

The significant grouping of cases seen in figures 3-6 is evidence that the true incubation period of the epidemic disease has a range from 2 to 4 weeks and a mode of 3.2 weeks as seen in figure 7. These peaks fit in with the two major peaks seen in figure 1, namely, at the borderline of May and June and the end of June. An incubation period of 3.2 weeks is similar to that found in the Middle East epidemic in 1942 among British troops that did not receive YFV (10).

The influence of YFV, nevertheless, needs to be accounted for. Although the onset of disease following vaccine has a considerable range, there is a relationship. Besides, it appears that certain lots of vaccine are tied up with jaundice a great deal more than others. It has been reported that the association tends to be an exclusive property of a certain few lots of vaccine (6), although this did not prove to be consistent in this study.

Lots of vaccine that are not considered to be "icterogenic" on a mass scale apparently have reputations in certain communities that rival the generally recognized "icterogenic" lots.<sup>5</sup>

<sup>5</sup> A recent, long paper (Sawyer, W. A., Meyer, K. F., Eaton, M. D., Bauer, J. H., Putnam, P., and Schwentker, F. F.: Jaundice in army personnel in the western region of the United States and its relation to vaccination against yellow fever (Part I). *Amer. Jour. Hyg.*, 39: 337-430, 1944 and (Parts II, III and IV), *Amer. Jour. Hyg.*, 40: 35, 1944) reports the widespread property of "icterogenicity" among the lots of YFV used by the Army. Among the 8,324 soldiers of the study, 63 lots of YFV were used. Of these, 24 lots were associated with 10 or more cases of jaundice. All remaining 39 lots were associated with at least one case. If the preepidemic attack rate of jaundice in the Army of 0.01 cases per 1000 per week be used as a baseline, it would appear that a total of approximately 3 cases would be the ex-

The considerable variability in "incubation period" attributed to vaccine noted in Brazil and in our own epidemic among different lots of vaccine does not favor the etiological character of vaccine in jaundice. Clinically identical jaundice has occurred among American troops in epidemic proportion in the Pacific island area as long as 9 to 13 months after YFV administration (9).

Although the YFV appears not to have had essential etiological significance, it seems to have played a real part in the background of the epidemic. This rôle has made it possible to predict, occasionally, where and when an epidemic of jaundice was likely to take root. Such predictions could not differentiate between the factors of etiology and susceptibility in an epidemic. Precisely what kinds of factors affect susceptibility in one or more individuals are generally obscure, yet probably form part of any epidemic background. Biological material contained in some YFV may have been a factor in the precipitation of epidemic jaundice. It is not unlikely that the physicochemical nature of homologous blood protein may become altered and, consequently, affect the equilibrium of relative immunity in its recipients. Such affects could vary from one lot of vaccine to another depending upon unpredictable and uncontrolled properties or events in the source, preparation and shipping of vaccine. It is interesting that the presence of human material in vaccines and serums associated with epidemics of jaundice has been a common factor frequently (1-6), but not invariably (7-8).

We are acquainted with the importance of exposure to inclement weather in the precipitation of respiratory infections (12). Herpes simplex is prone to break out in individuals with naturally or artificially induced fever (13). These are among the common examples of precipitation of latent disease by non-specific factors. Several similar and familiar experiences have been encountered in the laboratory from time to time. Epidemics may be initiated through stimulation of latent infection in certain foci, and transmission may proceed from such foci after a proper incubation period.

The likely alteration of relative immunity in the inoculated population of Camp "Baker" may have attained a high level about 6 to 9 weeks after inoculation with YFV. The mean incubation period of 3.2 weeks may have fol-

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pectancy during the 30 weeks of the epidemic among the 8,324 soldiers. If "icterogenicity" of YFV is considered to be primarily responsible for the epidemic attack rate of jaundice, only a maximum of 3 lots (one case per lot) out of 63 may be considered free of "icterogenicity" and 60 lots to have "icterogenicity" to some degree.

The level of significant "icterogenicity" appears to be entirely arbitrary. It would appear difficult to define "icterogenicity" in terms of occasionally contaminated vaccine in the face of its alleged universal presence.

It is noteworthy that figures 1, 2, 3, 4, 5, 8, 9, 13 and 14 in the same paper contain graphs with peaks about 2 to 4 weeks apart. Although the peaks are not striking individually, the consistent appearance is interesting.

Figures 11 and 13 are based on cases rather than percentage of cases among individuals probably unvaccinated or vaccinated with YFV of low "icterogenicity." The cases arising out of the relatively small proportion of soldiers remaining unvaccinated during the epidemic period may represent a high attack rate. Such evidence suggests that a high attack rate is not necessarily dependent upon "icterogenicity" of YFV.

lowed attainment of susceptibility. The epidemic started about 9 weeks and reached a peak 12 weeks after inoculation. Subsequent waves appear to have followed at 3 to 4 week intervals.

Sporadic prevaccine cases of jaundice plus the multiple occurrence of cases in particular barracks during the first wave suggest that carriers may disseminate the causative agent focally and that disease may appear in those foci under the influence of a precipitating factor (12).

The difficulty in isolating an etiological agent from jaundice leaves open a possibility that there may be more than one etiological agent operative in these cases regardless of their clinical pathological identity. One example of such a possibility is seen in influenzal viruses A and B. Multiplicity of etiology in the epidemic, however, does not justify the point of view that an essential etiological agent may have been present in the YFV.

The widespread use of human serum and other materials that may affect immune processes suggests a need for investigation into the causes of such effects.

#### SUMMARY

1. Spatial and temporal grouping of cases of jaundice in certain barracks and units among troops having received common YFV at approximately the same time points towards a communicable disease rather than one caused by "icterogenic" vaccine.

2. Similar grouping of cases in units that received mildly "icterogenic" YFV at an earlier period tends to substantiate communicability.

3. A 36-fold increase in attack rate in the unvaccinated population over the preepidemic rate during the major epidemic adds to the probability of communicability of jaundice.

4. There is significant evidence of an incubation period ranging from 2 to 4 weeks with a mode and mean of 3.2 weeks, a standard deviation of  $\pm 0.72$ , and a coefficient of variation of 22.5 per cent.

5. YFV used on certain Army posts appears to have had the property of predisposing its recipients to the communicable disease referred to as jaundice. The property varied in degree among the several lots of YFV.

6. The mode of transmission of jaundice is not ascertained but the common finding of personal association suggests dissemination by droplet or physical passage from hand to mouth. Flies may act as mechanical vectors. Carriers and individuals, inapparently infected, probably play important rôles in the epidemiology of jaundice.

7. The interval of time between administration of YFV and the epidemic appearance of jaundice cannot be called an "incubation period" properly since it is not directly related to the incubation of an infectious agent.

8. It is likely that all cases of jaundice at Camp "Baker", whether they did or did not receive YFV, had a common etiology although proof must await isolation of an etiological agent and the establishment of a specific diagnostic test.

I wish to express gratitude to Colonel Thomas J. Leary and Lieutenant Long of his staff for their excellent cooperation in the field study, and to Jane Evans

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# STUDIES ON SUSCEPTIBILITY OF MARSUPIALIA TO DIFFERENT STRAINS OF YELLOW FEVER VIRUS<sup>1</sup>

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During the years 1934 to 1940 a progressive epidemic wave of jungle yellow fever swept through the central and southeastern states of Brazil, being prevalent in most of the forested areas of these states. Presumably it reflected an epizootic, transmitted by blood-sucking arthropods by which man became infected when working or traveling through these forested areas.

As it was important to ascertain which animals were implicated in maintaining the virus in the forest, a systematic search for susceptible animals was initiated by several workers. Before 1934 it had been demonstrated by Davis (1, 2, 3, 4) that certain species of South American primates, including marmosets, were susceptible to yellow fever virus. Bugher *et al.* (5), in 1941, published a report on the susceptibility of Marsupialia to yellow fever virus. Recently Bates (6) reported experiments performed with marsupials, testing the susceptibility of two species.

The scope of this paper is to report additional observations on the susceptibility of Marsupialia to different strains of yellow fever virus.

## MATERIAL AND METHODS

### *Animals*

All of our animals, with two exceptions, were captured by our field units in areas in which yellow fever had been reported previously. The exceptions were the animals taken in the Tijuca woods (Federal District) and in the Baixada Fluminense, both in the vicinity of Rio de Janeiro. Captures were made either during the epidemic, as in the state of Espírito Santo, or a year or more afterwards, as in the other areas. For the most part young animals were used in the tests for susceptibility.

A list of the various species studied, their common names, and the locality from which specimens were obtained is given below:<sup>3</sup>

1) *Didelphis marsupialis* L.—common opossum. Captured in the Tijuca woods (Federal District), Mangaratiba and Teresópolis (State of Rio de Janeiro), Além Paraíba (State of Minas Gerais), and in the State of Espírito Santo, south of the Rio Doce.

<sup>1</sup> The work on which these observations are based was carried out under the auspices of the Serviço de Estudos e Pesquisas sobre a Febre Amarela (Yellow Fever Research Service), which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

<sup>2</sup> From the Laboratory of the Serviço de Estudos e Pesquisas sobre a Febre Amarela, Rio de Janeiro, Brazil.

<sup>3</sup> We are indebted to João Moojen, of the Museu Nacional, Rio de Janeiro, for the identification of most of the animals used.

2) *Didelphis paraguayensis* Oken.—white-eared common opossum. Captured at Pouso Alto, southern Goiaz.

3) *Metachirops opossum* (L.)—gray masked opossum. Captured in Mangaratiba, São João Marcos, and the Baixada Fluminense (State of Rio de Janeiro), and in Além Paraíba (State of Minas Gerais).

4) *Metachirus nudicaudatus* (E. Geoff.)—brown masked opossum. Captured in Mangaratiba (State of Rio de Janeiro), Tijuca woods (Federal District), Além Paraíba (State of Minas Gerais), and in the State of Espírito Santo, south of the Rio Doce.

5) *Caluromys philander* (L.)—woolly opossum. Captured in the Tijuca woods (Federal District), and Teresópolis (State of Rio de Janeiro).

6) *Marmosa cinerea* (Temminck)—murine opossum. Captured at Ilhéus (State of Bahia).

7) *Marmosa incana* (Lund)—murine opossum. Captured at Além Paraíba (State of Minas Gerais).

All of these animals were satisfactorily maintained under laboratory conditions. They were fed on meat, oranges, bananas, and our standard mouse food. Some difficulty was encountered in keeping murine opossums alive, as they are rather delicate animals. However, when newborn white mice and larva of the meal worm (*Tenebrio sp.*) were added to the diet, these animals could be maintained alive for several months.

#### *Methods of infection*

Intraperitoneal inoculation of the pantropic strains of yellow fever virus was employed at the beginning of the experiments, but this route was abandoned shortly. The majority of the animals studied were inoculated subcutaneously. In some instances, for the sake of comparison, other routes were employed. Intracerebral inoculations were made through a small hole bored in the skull, and the virus suspensions were injected into the midbrain. For intradermal inoculation the ventral surface of the thorax was shaved, and 0.5 ml. of the virus suspension was distributed over a scarified area, measuring 15 mm. in diameter, and was carefully rubbed in. For intranasal instillation, 0.5 ml. of the inoculum was introduced into each nostril by means of a syringe and blunt needle, while the animals were under light ether anesthesia.

#### *Virus strains employed*

In most of our experiments the J.Z. strain, isolated in 1937 in the State of Mato Grosso, or the O. C. strain, isolated in the State of Espírito Santo in 1940, was used. These strains were isolated in and passaged solely through rhesus monkeys (7). In two experiments the jungle strains M.D. and J.A., isolated and passaged in mice (3rd mouse passage), were employed. The Colombian jungle strain Martinez (5, 7), was used in some experiments with *D. marsupialis*. The Asibi strain was employed when in its 39th to 43rd rhesus passage. In one experiment the 17th rhesus passage was used.

Besides these pantropic strains, the French neurotropic strain was employed in its 520th to 575th mouse passage.



*Tests for susceptibility<sup>4</sup>*

Most of the animals under study were inoculated with small doses of virus. The virus inoculum was titrated in tenfold dilutions in 10 per cent normal monkey serum saline, and each dilution was inoculated intracerebrally in 0.03 ml. amount into each of a group of 6 or 12 albino Swiss mice. In general, the surviving mice, whether injected with the virus inoculum or with material from inoculated marsupials, were "challenged" by a intracerebral inoculation of  $\pm 500$  LD<sub>50</sub> of French neurotropic virus. The virus titers were calculated on the basis of a combined 50 per cent mortality and immunity, end point.

The inoculated marsupials were bled from the heart on successive days to test for the presence of circulating virus. The serum from these bleedings was inoculated intracerebrally in 0.03 ml. amounts into six albino Swiss mice of 17 to 35 days of age. The undiluted serum of *Didelphis* and *Metachirops* was toxic when inoculated intracerebrally into mice, and was, therefore, diluted 1:3 or 1:5 in saline. In the case of *Marmosa* and the very young animals of other species, whole blood was used. This was inoculated immediately into mice with the same syringe and needle which had been used for bleeding.

After a period of about 30 days the test animals were bled to determine whether neutralizing antibodies were found in their serum. Sera obtained prior to inoculation (except in the case of the newborn or pouch-young animals) served as controls and were tested at the same time by the intraperitoneal test in young mice (8). However, in certain experiments with *D. marsupialis* infected with the Martinez virus strain, *Marmosa incana*, and *M. cinerea*, the intracerebral neutralization test was employed (9, 10). None of the animals described here were considered to be immune prior to the test inoculation.

## EXPERIMENTAL

*Didelphis marsupialis* L.

The common opossum is prevalent in all parts of southeastern Brazil. This ubiquitous animal is found in forest and field and frequently visits houses to seek food or shelter. A total of 162 individuals was used. Of these, 82 were adults, 27 were juveniles, and 53 were newborn; the latter were still being carried in the pouch.

All the juvenile, newborn, and 48 of the adult opossums were tested for circulating virus, and the survivors were bled later for immunity. Table 1 summarizes these experiments. Of the 48 adults tested, only one was shown to circulate virus in the blood stream. This animal had been inoculated with  $4.8 \times 10^2$  LD<sub>50</sub> of the Martinez strain, and virus circulated on the sixth to the eighth day. Of the 27 juveniles tested only one was found to circulate virus. This one had been inoculated with  $1 \times 10^2$  LD<sub>50</sub> of the Asibi strain, and virus was recovered from the third to the sixth day after inoculation.

<sup>4</sup> A susceptible species is defined in this paper as one in which the majority of the inoculated animals (a) circulated virus for two or more consecutive days, after an initial period of at least 24 hours during which no virus was demonstrable in the blood, (b) subsequently developed neutralizing antibodies.

Of the 26 newborn opossums, none circulated virus after inoculation with either of two jungle strains of yellow fever virus. In five out of 27 injected with Asibi strain, virus was demonstrable, but on the second day only. The surviving animals were tested later for immunity and only four, all inoculated with J.Z. virus, were found to possess neutralizing antibodies.

These experiments also indicate that only a small fraction of the inoculated opossums develop a humoral immunity. Thus, out of 103 surviving animals of all age groups, only 13 showed circulating antibodies. Of the three adult opos-

TABLE 1

*Circulation of virus in Didelphis marsupialis after the administration of various strains of yellow fever virus*

ANIMAL		VIRUS INOCULUM			RESULTS			
Number tested	Age	Strain	Range of LD <sub>50</sub> of virus inoculated	Route*	Days tested for virus	Number circulating virus	Days on which virus circulated	Immunity ratio†
11	Adult	M.D.	$3 \times 10^1$	I.P.	2nd to 12th	0		0/11
7	Adult	J.A.	$8.2 \times 10^1$	I.P.	2nd to 12th	0		0/7
4	Adult	F.N.	$1.5 \times 10^0$ to $1.5 \times 10^6$	I.C.	1st to 7th	0		1/3
3	Adult	F.N.	$3 \times 10^1$ to $3 \times 10^5$	I.N.	1st to 7th	0		0/2
23	Adult	Martinez	$9 \times 10^0$ to $4.8 \times 10^3$	S.C.	1st to 7th and 8th	1	6th to 8th	2/20
12	Juvenile	Asibi	$1 \times 10^2$ to $3 \times 10^3$	S.C.	1st to 7th and 2nd to 5th	1	3rd to 6th	2/8
7	Juvenile	J.Z.	$8.2 \times 10^5$	S.C.	2nd to 5th	0		1/5
8	Juvenile	O.C.	$9 \times 10^3$	S.C.	2nd to 5th	0		3/8
27	Newborn	Asibi	$5 \times 10^1$ to $1.2 \times 10^6$	S.C.	1st to 7th and 10th	5	2nd	0/14
14	Newborn	J.Z.	$3.7 \times 10^3$	S.C.	1st to 5th	0		4/13
12	Newborn	O.C.	$4.3 \times 10^3$	S.C.	1st to 5th	0		0/12

\* Route: I.P. = intraperitoneal, I.C. = intracerebral, I.N. = intranasal, S.C. = subcutaneous.

† Numerator = number of animals developing antibodies; denominator = total number of animals surviving the experiment.

sums which became immune, one had been inoculated intracerebrally with a large amount of French neurotropic virus, and two had been inoculated subcutaneously with the Martinez strain. Six of 21 juvenile opossums became immune, and only a small percentage of the newborn opossums showed a humoral immunity after the test inoculation. It should be noted that these newborn animals were not bled prior to the virus injection.

In order to compare the immunity response of the adult opossum to infections produced by injections other than by the subcutaneous route, a few additional experiments were performed. As can be seen from table 2 the route of inoculation seemed to be of no importance. Only the animals which received a large amount of virus developed a humoral immunity.

From these experiments we conclude that adults of the species *D. marsupialis* are resistant to the Asibi strain, the Brazilian jungle strains, the Martinez strain, and the French neurotropic strain. Only one out of 23 inoculated with the Martinez strain, a strain of Colombian origin which was used extensively in the experiments of Bugher *et al.*, circulated virus. Adult opossums, as a rule, required the inoculation of a large amount of virus in order to develop circulating antibodies. Young and juvenile opossums, while still in the pouch, were tested against the Asibi, J.Z., and O.C. strains; but, with the exception of a minority inoculated with the Asibi strain, they failed to circulate virus on the days tested. The immunity response of these young opossums was poor.

TABLE 2  
*Reaction of Didelphis marsupialis after the administration of yellow fever virus by different routes*

ANIMALS		VIRUS INOCULUM			RESULT
Number tested	Age	Strain	LD <sub>50</sub> inoculated	Route*	Immunity ratio†
6	Adult	F.N.	$3 \times 10^8$	I.N.	6/6
11	Adult	Asibi	$2.2 \times 10^8$	I.P.	11/11
5	Adult	Asibi	$7.5 \times 10^3$	I.P.	0/5
3	Adult	Asibi	$3 \times 10^3$	I.D.	0/3
3	Adult	F.N.	$9.5 \times 10^6$	S.C.	3/3
3	Adult	F.N.	$9.5 \times 10^6$	I.C.	3/3
3	Adult	F.N.	$9.5 \times 10^6$	I.D.	3/3

\* Route: I.N. = intranasal, I.P. = intraperitoneal, I.D. = intradermal, S.C. = subcutaneous, I.C. = intracerebral.

† See table 1.

### *Didelphis paraguayensis* Oken

The white-eared opossum is at home in the southcentral states of Brazil, where yellow fever was present from 1934 to 1937. At the beginning of 1942 a few of these animals were captured in southern Goiaz. These were tested by the subcutaneous inoculation of the Asibi strain and also the J.Z. and O.C. jungle strains. They were bled from the heart on the second and the seventh day. Two adults were inoculated with  $6 \times 10^5$  LD<sub>50</sub> of Asibi virus. One failed to show virus in circulation but developed partial immunity. The other had a small amount of virus in the blood stream, on the second day only, and developed neutralizing antibodies.

Of six young animals inoculated with moderate doses of either the O.C. or J.Z. strains, none circulated virus and none developed neutralizing antibodies.

*Didelphis paraguayensis* was found to be resistant to the strains used.

### *Metachirops opossum* (L.)

The grey masked opossum occurs in forests and second growth. Thirty-six adults of this species and 32 young still in the pouch were tested.

Thirty-four of the adults were tested against various strains of yellow fever

virus. The results of this test are presented in table 3. Fourteen were inoculated with the high passage Asibi strain and five showed circulating virus. Of

TABLE 3

*Occurrence of circulating virus in adult Metachirops opossum after subcutaneous administration of yellow fever virus*

ANIMAL NUMBER	VIRUS INOCULUM		CIRCULATING VIRUS IN FOLLOWING DAYS*								NEUTRALIZATION TEST†			
	Strain	LD <sub>50</sub>	1	2	3	4	5	6	7	8	Pre-inoculation		Post-inoculation	
											PR	AST	PR	AST
1	Asibi high	$3.6 \times 10^4$	0/6	<u>5/5</u>	<u>2/3</u>	0/3	0/5	0/6	0/2	Died	1/6	6.50		
2	Asibi high	$3.6 \times 10^4$	0/5	0/6	0/4	0/4	0/5	0/4	0/6		<u>3/6</u>	7.66	0/6	5.83
3	Asibi high	$2.5 \times 10^4$	0/4	0/5	0/6	0/5	0/6	0/5	0/5	0/6	0/6	4.00	<u>5/6</u>	9.00
4	Asibi high	$2.5 \times 10^4$	0/5	0/5	0/6	0/5	0/6	0/6	0/2	0/5	0/6	3.50	0/6	3.67
5	Asibi high	$2.5 \times 10^3$	0/6	0/5	<u>5/5</u>	0/6	0/4	0/6	0/6	0/5	1/6	4.83	<u>6/6</u>	10.0
6	Asibi high	$2.5 \times 10^3$	0/4	0/4	0/6	0/5	0/4			Died	0/6	4.33		
7	Asibi high	$7.5 \times 10^2$	0/5	0/4	<u>3/6</u>	<u>2/6</u>	<u>6/6</u>	0/5		Died	1/12			
8	Asibi high	$7.5 \times 10^2$	0/5	0/6	<u>1/6</u>	0/6	<u>6/6</u>	0/5	0/6	0/5	4/12		<u>11/12</u>	
9	Asibi high	$3.6 \times 10^2$	0/6	0/5	<u>5/6</u>	<u>5/6</u>	0/6	0/5	0/4		0/6	5.33	<u>6/6</u>	10.0
10	Asibi high	$3.6 \times 10^2$	0/6	0/6	0/6	0/6	0/6	0/5	0/6		0/6	5.16	0/6	5.50
11	Asibi high	$2.5 \times 10^2$	0/3	0/3	0/6	0/3	0/6	0/6	0/6	0/4	0/6	4.00	0/6	4.00
12	Asibi high	$2.5 \times 10^2$	0/6	0/4	0/4	0/4	0/5	0/5	0/6	0/0	0/6	4.00	0/6	4.00
13	Asibi high	$7.5 \times 10^0$	0/3	0/3	0/5	0/5	0/4	0/4	0/4	0/3	0/12		1/12	
14	Asibi high	$7.5 \times 10^0$	0/5	0/2	0/6	0/6	0/6	0/6	0/6	0/4	1/12		1/12	
15	Asibi low	$1.9 \times 10^3$	0/5	0/4	0/5	0/6	0/6	0/5	0/5	0/6	0/6	3.83	0/6	4.00
16	Asibi low	$1.9 \times 10^3$	0/5	<u>2/5</u>	<u>6/6</u>	<u>2/4</u>	0/6	0/6	0/5	0/4	0/6	4.17	<u>6/6</u>	10.0
17	Asibi low	$1.9 \times 10^2$	0/6	0/6	0/6	0/4	0/6	0/6	0/6	0/5	0/6	4.33	0/6	3.83
18	Asibi low	$1.9 \times 10^2$	0/5	0/2	<u>6/6</u>	<u>1/5</u>	0/6	0/6	0/5	0/5	0/6	4.17	1/6	5.67
19	Asibi low	$1.9 \times 10^0$	0/6	0/6	0/6	0/4	0/6	0/6	0/6	0/6	0/6	3.67	0/6	4.33
20	Asibi low	$1.9 \times 10^0$	0/6	0/5	0/6	0/6	0/6	0/5	0/6	0/6	0/6	3.87	0/6	4.00
21	O.C.	$2.2 \times 10^5$	0/3	0/6	0/6	<u>2/6</u>	0/6	0/6	0/5	0/6	0/6	4.50	<u>6/6</u>	10.0
22	O.C.	$2.2 \times 10^5$	0/5	0/6	0/5	0/6	0/4	0/5	0/3	0/6	0/6	4.67	0/6	5.33
23	O.C.	$2.2 \times 10^3$	0/5	0/5	0/4	0/4	0/6	0/4	0/6	0/4	0/6	4.67	0/6	4.67
24	O.C.	$2.2 \times 10^3$	0/3	0/5	0/5	0/6	0/2	0/3	0/2	0/5	1/6	5.33	0/6	4.50
25	O.C.	$2.2 \times 10^1$	0/5	0/6	0/6	0/6	0/5	0/6	0/3	0/5	1/6	6.00	1/6	5.17
26	O.C.	$2.2 \times 10^1$	0/4	0/6	0/6	0/6	0/6	0/6	0/3	0/6	0/6	4.83	0/6	5.00
27	J.Z.	$2 \times 10^4$	0/4	0/5	0/5	0/6	0/4	0/3		Died	0/6	5.16		
28	J.Z.	$2 \times 10^4$	0/4	0/5	0/4	0/5	0/5	<u>1/2</u>	<u>3/5</u>		0/6	5.33	<u>3/6</u>	8.00
29	J.Z.	$1.4 \times 10^3$	0/5	0/6	0/6	0/6				Died	0/6	4.50		
30	J.Z.	$1.4 \times 10^3$	0/5	0/6	1/5	0/5	0/5	0/6	0/6	0/4	<u>4/6</u>	8.17	0/6	5.00
31	J.Z.	$2 \times 10^2$	0/3	0/4	0/4	0/1	0/4	0/4	0/5		0/6	4.83	0/6	5.16
32	J.Z.	$2 \times 10^2$	0/3	0/4	0/6	0/4	0/6	0/4	0/5		1/6	6.16	6/6	10.0
33	J.Z.	$1.4 \times 10^1$	0/4	0/6	0/6	0/6				Died	1/6	5.50		
34	J.Z.	$1.4 \times 10^1$	0/5	0/5	0/6	0/6	0/4	0/3	0/6	0/4	1/6	5.50	0/6	4.33

\* Denominator = number of mice inoculated; numerator = number of mice dead or immune.

† PR = protection ratio; number of mice protected over total inoculated. AST = average survival time of inoculated mice.

these, three survived and developed neutralizing antibodies. Six others were tested against the low passage Asibi strain. Two circulated virus, but only the

one which showed the largest amount of virus in the blood stream acquired humoral immunity.

Six others were inoculated with the O.C. virus strain. One of two which had been inoculated with  $2.2 \times 10^5$  LD<sub>50</sub> showed a very small amount of virus in the circulation and was the only animal to develop neutralizing antibodies.

Eight animals of this species were tested against the J.Z. strain, and again one had virus in the blood stream, but only on the 7th day, which was the last day of the test. This one became immune.

Two adult gray masked opossums were inoculated intracerebrally with over  $7 \times 10^6$  LD<sub>50</sub> of French neurotropic virus in its 526th mouse passage. The animals were under observation for 20 days, and no sign of encephalitis was observed during this period. On the 20th day both animals were bled to death. The skulls were examined, and in one the perforation through the cranium was still visible. Judging by its location the inoculation had been made into the mid-brain. Both animals had developed circulating antibodies by the 20th day.

Thirty-two young opossums, still being carried in the pouch, were inoculated subcutaneously with various strains of yellow fever virus. The larger animals were bled at intervals, but the smaller individuals, which could not be bled, were sacrificed by decapitation, one on each day, and the brain was removed under aseptic conditions. The skin over the thorax was dissected off, and the heart, lung, and liver were removed. With still smaller animals the thorax, without the skin but containing its viscera, was saved. After weighing, the different parts or organs were ground up individually in a mortar, without abrasive, and were made up to a 10 per cent suspension in normal monkey serum-saline. The suspension was spun in an angle centrifuge at 3,500 r.p.m. for 30 minutes, and the supernatant was tested for virus. Table 4 shows the results obtained. Against all expectations virus was recovered only in six instances. However, it is of interest that, again, more individuals showed virus in the blood stream or in the viscera after the administration of the Asibi than with other virus strains. No virus was found in the brains of the animals tested. The immunity response was poor. Of two which had virus in the blood stream on the third day, only one developed circulating antibodies.

*Metachirops opossum* should be considered as resistant to the virus strains used, especially to the jungle virus strains, although a small percentage of the inoculated animals showed virus in circulation.

The immunity response to the strains employed was poor. Only a portion of the animals that had virus in circulation developed neutralizing antibodies.

#### *Metachirus nudicaudatus* (E. Geoff.)

The brown masked opossum is found in the forest and second growth. It is prevalent in all parts of the central and southeastern states of Brazil.

Forty-four adult opossums of this species were used. Of these, forty-two were inoculated subcutaneously with pantropic yellow fever virus strains, and tests for virus in the blood stream were made. Table 5 summarizes these experiments, and table 6 shows the details of one single experiment, giving the results of the daily bleedings and of the neutralization tests.

Two of the opossums were inoculated intracerebrally with over  $7 \times 10^6$  LD<sub>50</sub> of French neurotropic virus. None developed encephalitis during a 20-day ob-

TABLE 4

*Reaction of pouch-young Metachirops opossum after subcutaneous inoculation of yellow fever virus*

ANIMALS		VIRUS INOCULUM		DAYS TESTED FOR VIRUS	ORGANS INOCULATED INTO MICE*	RESULTS		
Number tested	Average weight	Strain	LD <sub>50</sub>			No. with virus	Days in which virus was present	Immunity ratio†
6	grams 3.45	Asibi	$1 \times 10^2$	one each from 1st to 6th	Brain L.L.H.	0 2	3rd and 5th	
4	27.0	Asibi	$9.6 \times 10^3$	2 on alternate days, 1st to 5th	Blood	3	2nd and 3rd	1/2
8	1.83	O.C.	$5.6 \times 10^2$	1 each day on 1st to 8th	Brain Thorax	0 1	6th	
7	28.0	O.C.	$2 \times 10^5$	3 and 4 alternating on 1st to 5th	Blood	0		1/5
7	23.0	J.Z.	$3.5 \times 10^3$	4 and 3 alternating on 1st to 5th	Blood	0		2/6

\* L.L.H. = pool of liver, lung, and heart; thorax = thorax plus its viscera.

† See table 1.

TABLE 5

*Reaction of Metachirus nudicaudatus after subcutaneous injection of yellow fever virus*

NUMBER OF ANIMALS TESTED	VIRUS INOCULUM			RESULTS			
	Strain	Range of LD <sub>50</sub>	LD <sub>50</sub> producing circulating virus	Days tested for virus	No. with circulating virus	Days in which virus was present	Immunity ratio*
10	Asibi	$0.95 \times 10^0$ to $9.5 \times 10^4$	$1.3 \times 10^1$ to $9.5 \times 10^4$	1st + 2nd to 7th	6	2nd to 6th	5/10
19	J.Z.	$1.2 \times 10^0$ to $8 \times 10^4$	$1.2 \times 10^1$ to $8 \times 10^4$	1st + 2nd to 7th	7	2nd to 6th	6/12
13	O.C.	$5 \times 10^1$ to $3.8 \times 10^5$	$5 \times 10^1$ to $3.8 \times 10^5$	1st + 2nd to 7th	13	1st to 6th	12/12

\* See table 1.

servation period. When sacrificed on the 20th day, one still showed evidence that the injection had been made into the midbrain. Both developed circulating antibodies.

In the light of these experiments, *Metachirus nudicaudatus* should be considered as a susceptible species. It could be infected with the Asibi, J.Z., and O.C. strains of yellow fever virus, but was much more susceptible to the O.C. strain than to the others. The incubation period was short, and virus persisted in the blood stream for three or more days. However, the immunity response to the virus strains employed was weak, and it cannot be compared to that developed in primates. After the inoculation of a small amount of Asibi or J.Z. virus, there were animals which, in spite of having circulated virus in the blood stream for several days, did not show any significant difference between serum drawn prior to the test and that secured on the 30th day of the experiment. It is presumed that these animals developed some degree of immunity, but that this was

TABLE 6  
Detail of Table 5. Reaction of *M. nudicaudatus*.

ANIMAL NUMBER	VIRUS INOCULUM		CIRCULATING VIRUS IN FOLLOWING DAYS*								NEUTRALIZATION TEST†			
											Preinoculation		Postinoculation	
	Strain	LD <sub>50</sub>	1	2	3	4	5	6	7		PR	AST	PR	AST
1	Asibi	$1.3 \times 10^2$	0/6	$\frac{1}{6}$	$\frac{6}{6}$	$\frac{3}{6}$	0/6	0/6	0/6	0/6	0/6	4.50	$\frac{2}{6}$	7.0
2		$1.3 \times 10^1$	0/6	$\frac{1}{6}$	$\frac{2}{6}$	$\frac{5}{5}$	$\frac{1}{6}$	0/6	0/6	0/5	4.80	$\frac{0}{5}$	4.80	
3		$1.3 \times 10^0$	0/6	0/6	0/6	0/5	0/6	0/6	0/6	0/6	4.66	0/6	4.0	
4	J.Z.	$1.2 \times 10^2$	0/6	$\frac{5}{6}$	$\frac{6}{6}$	$\frac{5}{5}$	0/6	0/5	0/6	0/6	4.50	$\frac{1}{6}$	4.83	
5		$1.2 \times 10^1$	0/5	$\frac{3}{6}$	$\frac{6}{6}$	$\frac{6}{6}$	$\frac{5}{6}$	$\frac{2}{6}$	0/6	0/6	4.00	Died		
6		$1.2 \times 10^0$	0/6	0/6	0/5	0/6	0/6	0/5	0/6	1/6	5.50	0/5	4.80	
7	O.C.	$5 \times 10^2$	$\frac{1}{6}$	$\frac{5}{6}$	$\frac{5}{6}$	$\frac{6}{6}$	0/6	0/6	0/6	0/6	4.33	$\frac{5}{6}$	9/66	
8		$5 \times 10^2$	0/6	$\frac{5}{6}$	$\frac{6}{6}$	$\frac{5}{6}$	0/5	0/5	0/5	0/6	4.50	$\frac{4}{6}$	8/33	
9		$5 \times 10^1$	0/6	0/6	$\frac{3}{6}$	$\frac{6}{6}$	$\frac{5}{6}$	0/6	0/6	0/6	4.33	$\frac{6}{6}$	10.0	
10		$5 \times 10^1$	0/6	$\frac{1}{6}$	$\frac{5}{6}$	$\frac{3}{6}$	$\frac{2}{6}$	0/6	0/6	0/4	4.50	$\frac{3}{6}$	8/50	

\* Denominator = number of mice inoculated; numerator = number of mice dead or immune.

† PR = Protection ratio; number of mice protected over total inoculated. AST = Average survival time of inoculated mice.

of short duration. All 13 animals tested against the O.C. strain had circulating antibodies on the 30th day of the experiment. However, a certain number of sera had only a partial neutralizing capacity.

#### *Caluromys philander* (L.)

The woolly opossum is a semi-arboreal animal, sleeping during the day in some cavity or nest above the ground. Twelve individuals of this species were tested.

In a preliminary experiment each of four animals was inoculated with  $7 \times 10^1$  to  $7 \times 10^3$  LD<sub>50</sub> of Asibi virus and tested for circulating virus from the first day post inoculation. Virus was recovered in all four on the second day, but three died from bleeding accidents on this and the following day. One survived repeated bleedings, showing virus in the blood stream for four consecutive days and developing neutralizing antibodies.

Table 7 summarizes the following experiment.

Opossums 5, 6, and 7 were inoculated subcutaneously with the O.C. strain. They circulated virus on three consecutive days and developed neutralizing antibodies.

Animals 8, 9, 10, and 11 were inoculated subcutaneously with the J.Z. strain. None circulated virus and, with the exception of opossum no. 8, none showed a humoral immunity. Opossums 9 and 10 were subsequently inoculated with the O.C. strain, and no. 11, together with no. 12, a control animal, was inoculated with the Asibi strain. All circulated virus and developed neutralizing antibodies.

From these limited experiments it is concluded that *C. philander* is not susceptible to the J.Z. strain of yellow fever virus but is readily susceptible to two

TABLE 7

*Reaction of Caluromys philander to subcutaneous inoculation of yellow fever virus*

ANIMAL	VIRUS INOCULUM			CIRCULATING VIRUS IN FOLLOWING DAYS*									NEUTRALIZATION TEST†			
	Strain		LD <sub>50</sub>	1	2	3	4	5	6	7	8	9	Pre-inoculation		Post-inoculation	
	1st inoculum	2nd inoculum											PR	AST	PR	AST
5	O.C.		1.1 × 10 <sup>4</sup>	0/6	<u>6/6</u>	<u>6/6</u>	<u>6/6</u>	0/6	0/5	0/6	0/5		0/6	4.83	2/6	6.50
6	O.C.		4 × 10 <sup>2</sup>		0/5	<u>6/6</u>	<u>6/6</u>	<u>6/6</u>	0/5	0/6			0/6	4.83	<u>5/6</u>	9.38
7	O.C.		4 × 10 <sup>2</sup>		0/6	<u>6/6</u>	<u>6/6</u>	<u>5/6</u>	0/5	0/1			0/6	5.00	<u>5/6</u>	9.17
8	J.Z.		1.7 × 10 <sup>5</sup>	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/5		1/6	5.50	<u>3/6</u>	8.00
9	J.Z.		2.3 × 10 <sup>2</sup>		0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	4.33	0/6	4.67
9		O.C.	1.1 × 10 <sup>5</sup>	0/6	0/5	0/6	<u>6/6</u>	<u>6/6</u>	<u>4/4</u>	0/6	0/6		0/6	4.67	<u>6/6</u>	10.0
10	J.Z.		8 × 10 <sup>2</sup>	0/6	0/6	0/4	0/6	0/4	0/6	0/5	0/6		0/6	4.33	0/6	4.67
10		O.C.	4 × 10 <sup>2</sup>		0/5	<u>3/4</u>	<u>6/6</u>	<u>6/6</u>	0/6	0/4			0/6	4.67	<u>4/6</u>	9.17
11	J.Z.		8 × 10 <sup>3</sup>		0/5	0/5	0/3	0/6	0/6	0/5			0/6	4.33	1/6	6.50
11		Asibi	1.4 × 10 <sup>3</sup>	0/6	0/6	<u>6/6</u>		0/6		0/6		0/6			<u>6/6</u>	10.0
12	Asibi		1.4 × 10 <sup>3</sup>	0/3	0/6	<u>6/6</u>		0/6		0/4		0/5	0/6	4.83	<u>4/6</u>	9.17

\* Denominator = number of mice inoculated; numerator = number of mice dead or immune.

† PR = protection ratio; number of mice protected over total inoculated. AST = average survival time of inoculated mice.

other yellow fever virus strains. None of these three strains can be differentiated by immunological reactions.

#### *Marmosa cinerea* (Temminck)

Eleven animals of this species were tested against the O.C. and J.Z. strains of yellow fever virus.

All these opossums were inoculated by the subcutaneous route and were bled for circulating virus on the second, fourth, sixth, and ninth day of the experiment. Six were inoculated, each with  $7.5 \times 10^2$  LD<sub>50</sub> of O.C. virus, and all but one showed variable amounts of virus in circulation. In one opossum virus could be demonstrated on the ninth day only, and the following day the animal was found dead, probably as the result of bleeding. Blood serum and brain and liver



suspensions of the dead animal were tested and virus was found in each instance, the highest concentration being in the serum. Three murine opossums survived up to the 30th day. One which had not circulated virus became immune. Of the two surviving animals which circulated virus, one failed to develop antibodies but the second became immune.

Five other animals of the species received  $1.5 \times 10^2$  LD<sub>50</sub> of J.Z. virus each. None circulated virus and none developed neutralizing antibodies.

*Marmosa cinerea* should also be considered a susceptible animal, at least to the O.C. virus strain.

#### *Marmosa incana* (Lund.)

This murine opossum, like the preceding species, is a semi-arboreal animal preferring for its habitat thick undergrowth in forests and cultivated land, in which it can build its nest and seek shelter and food.

Thirty *Marmosa incana* were tested against three strains of yellow fever virus. As these animals are fragile, weighing about 50 grams, many died during the course of the experiment from bleeding injury, and only 16 could be tested for an immune response. Only animals which survived at least three consecutive bleedings during the course of the susceptibility study are recorded here.

Six opossums were tested against the Asibi strain, each receiving an amount of virus ranging from  $1.4 \times 10^2$  LD<sub>50</sub> to  $6.8 \times 10^5$  LD<sub>50</sub>. All circulated virus for three consecutive days, with the exception of one which received the smallest virus dose and from which virus could be recovered on the third day only. Only four of the animals could be tested for immunity, and all of these were found to have circulating antibodies.

Ten animals were inoculated with the O.C. strain of virus. The amount of virus inoculated varied from  $5 \times 10^1$  LD<sub>50</sub> to  $3.8 \times 10^4$  LD<sub>50</sub>. All showed virus in the circulation. The ones which received the higher amount of virus showed an early and short period of virus circulation, namely two to three days, beginning on the second day of the experiment. The ones inoculated with the smallest amount of virus had a longer period of circulating virus, i.e. four or five consecutive days, beginning on the second or third day of the experiment. Only two could be tested for immunity, and both had developed circulating antibodies.

Fourteen murine opossums were inoculated with the J.Z. strain of virus. The virus dose inoculated into each varied from  $8 \times 10^1$  LD<sub>50</sub> to  $5 \times 10^4$  LD<sub>50</sub>. Only seven showed virus in the blood stream. As it was conceivable that virus could appear late in the circulation, five opossums were bled on alternate days up to the 13th day of the experiment. Table 8 gives the details of two such sets of experiments. It can be seen that five animals circulated virus. Two of the three animals receiving the largest amounts of virus had the longest period of circulation, but the third animal, receiving the same amount, failed to show either virus or antibodies in the blood stream. Animal no. 7 had circulating virus on the seventh day of the experiment and became immune. On the other hand, no. 8 circulated virus early, but when bled on the 30th day of the experiment was found to be without humoral immunity. Animal no. 9 reacted in the same way. It

would seem that inoculation of small amounts of virus in this species produces only a short-lived immunity.

In the light of these experiments *Marmosa incana* should be considered partially susceptible to the yellow fever virus strains employed.

TABLE 8  
*Susceptibility of Marmosa incana to the J.Z. strain of yellow fever virus*

ANIMAL NUMBER	LD <sub>50</sub> OF VIRUS INOCULATED	CIRCULATING VIRUS IN FOLLOWING DAYS										IMMUNITY RESPONSE
		1	2	3	4	5	6	7	9	11	13	
1	$5 \times 10^4$	0/6	2/6	5/6	6/6	6/6	0/5	0/6				+
2	$5 \times 10^4$	0/6	2/6	6/6	6/6	6/6	0/6	0/6				+
3	$5 \times 10^4$	0/6	0/6	0/6	0/6	0/6	0/6	0/6				-
4	$5 \times 10^2$	0/6	0/6	0/6	0/6	0/6	0/6	0/6				Died
5	$5 \times 10^2$	0/6	0/6	0/5	0/6	0/6	0/6	0/6				-
6	$8 \times 10^3$	0/4	0/6	0/5		0/6		0/6	0/6	0/6	0/5	-
7	$8 \times 10^3$	0/6	0/6	0/6		0/6	6/6	0/5	0/6	0/6	0/5	+
8	$8 \times 10^3$	0/5	3/5	6/6		0/6	0/6	0/5	0/5	0/5	0/6	-
9	$8 \times 10^1$	0/5	0/6	3/3		2/5	0/6	0/6	0/6	0/3	0/6	-
10	$8 \times 10^1$	0/6	0/6	0/6		0/6	0/6	0/6	0/6	0/4	0/6	-

#### DISCUSSION

These experiments have partially confirmed and extended the observations of Bugher *et al.* on marsupials. These authors tested the susceptibility of *Didelphis marsupialis*, *D. paraguayensis*, *Metachirops opossum*, (= *Philander opossum*), *Metachirus nudicaudatus*, *Caluromys laniger*, and *Marmosa sp.* As a result of the observations on these animal species the authors concluded that all of the species of Marsupialia tested were susceptible to the virus of yellow fever, in that, following small doses of virus administered subcutaneously, at least a portion of the animals of each species exhibited a rapid multiplication of the virus and the formation of specific antibodies. These authors suggested that marsupials might be of importance in the epidemiology of jungle yellow fever.

In the light of our experiments we conclude that *Didelphis marsupialis*, *D. paraguayensis*, and *Metachirops opossum* are resistant to the virus strains used. Only a few individuals of the above-mentioned species circulated virus, and in general the immunity response was poor. Newborn opossums behaved in a similar way—few circulated virus and only a small percentage developed a humoral immunity after injection. This is significant since, owing to the embryo-like state in which these animals are born, they should, theoretically, be more susceptible than adult animals. South American opossums are aplacental animals, and it is unlikely that the pouch-young animals can acquire passive immunity through the milk of the adult.

In general our results are similar to those presented by Bugher *et al.* and by Bates. In their experiments only a small percentage of individuals circulated virus and developed neutralizing antibodies. The first mentioned authors, however, came to the conclusion that these three species were susceptible to yellow

fever. Bates, on the contrary, concluded that *Metachirops opossum* was resistant to infection with the strains of yellow fever virus tested.

*Metachirus nudicaudatus* is susceptible to yellow fever virus, and our experiments are in accord with those presented by Bugar *et al.*, and by Bates.

*Caluromys philander*, *Marmosa incana*, and *M. cinerea* should also be considered as susceptible animals. The reaction of *C. philander* to the different strains of yellow fever virus is of special interest. It is susceptible to the O.C. and Asibi strains, but seems not to be susceptible to the J.Z. strain. When these animals that had been resistant to the J.Z. strain were reinoculated subsequently with either the O.C. or the Asibi strain, they proved to be susceptible, circulating virus promptly and later developing neutralizing antibodies. *M. cinerea* also circulates virus when inoculated with the O.C. strain but not when inoculated with the J.Z. strain.

This is of interest since it indicates that certain animal species may be completely indifferent to the inoculation of one given strain of yellow fever virus, but may be fully susceptible to another strain which is immunologically indistinguishable.

It seems, therefore, that the J.Z. strain reacts peculiarly in certain species of Marsupialia. On the other hand, the O.C. and the Asibi strain infect certain species of Marsupialia, such as *M. nudicaudatus*, *M. incana*, and *M. cinerea*. In view of this it is suggested that there may be still other yellow fever virus strains to which certain species of Marsupialia are more susceptible.

#### SUMMARY

1. Three hundred and thirty-five South American opossums, belonging to seven different species, were tested against several strains of yellow fever virus.

2. *Didelphis marsupialis*, *Didelphis paraguayensis*, and *Metachirops opossum* were found to be resistant to all of the virus strains used; very few opossums circulated virus, and, in general, the immunity response was poor. In addition, *M. opossum* was found to be resistant to intracerebral inoculation of French neurotropic virus.

3. *Metachirus nudicaudatus* and *Marmosa incana* were found to be susceptible to the virus strains employed. A great majority of the inoculated animals circulated virus, and nearly all of these developed a humoral immunity. However, *M. nudicaudatus* did not show any symptoms of encephalitis when inoculated intracerebrally with French neurotropic virus.

4. *Marmosa cinerea* and *Caluromys philander* were found to be partially susceptible to the virus strains used. Both species were resistant to the inoculation of the J.Z. virus strain but were found to be fully susceptible to the O.C. virus strain. *C. philander*, which failed to show circulating virus after injection of the J.Z. virus strain, promptly circulated virus when reinoculated with either the O.C. or the Asibi strain of virus.

5. Not only was it found that the individual species responded differently to the various virus strains employed, but also individuals of the same species reacted in different ways to the strains of virus used.

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# BROMELIAD MALARIA IN TRINIDAD, BRITISH WEST INDIES<sup>1</sup>

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## INTRODUCTION

The possibility that bromeliad-breeding anophelines of the subgenus *Kerteszia* might be involved in malaria transmission has been considered both in Brazil and in Trinidad since the beginning of the century, and much controversy has surrounded the subject. The importance in Trinidad of the bromeliad-breeder, *Anopheles (Kerteszia) bellator* D. & K., had been urged by both Urich (*vide* Boyce (1)) and de Verteuil (2) on several occasions before it was finally established in 1942 by Rozeboom and Laird (3), and Downs, Gillette, and Shannon (4).

In the present paper an account is given of the history of investigations and discussion on bromeliad malaria, along with a consideration of its ecological basis in Trinidad and the new control problems that arise.

The term "bromeliad malaria" has been selected to cover those cases where the vector is bromelicolous, since it serves to emphasize the unity these cases have in the uniqueness of the control problems that are presented.

## HISTORY OF INVESTIGATIONS ON BROMELIAD MALARIA

In 1903 Lutz published his paper "Waldmosquitos und Waldmalaria" (5), in which he described the epidemiological conditions that obtained during an outbreak of malaria among laborers working on the construction of a railroad in the mountain forests of São Paulo. Lutz found that ground-breeding anophelines were virtually absent, but that *Anopheles lutzi* Theobald (= *cruzi* D. & K.) was common; and he was forced to the conclusion that this bromeliad-breeding species was the vector. This conclusion rested purely on the epidemiological facts. Two years later, however, Galli Valerio (6) published a brief note illustrating an oocyst-infected mosquito stomach which was taken from a much damaged specimen collected in Paranagua, Brazil, and identified by himself as *Anopheles lutzi* Theobald.

Some years later Knab (7) criticized Lutz's conclusions and suggested that the epidemic of forest malaria which he had studied was a general recrudescence of latent infection due to the overexertion of the laborers involved. Knab did not believe that a forest anopheline was capable of transmitting human malaria, as it would, he suggested, bite man far too infrequently. He regarded *Anopheles lutzi* as only incidentally present in the area studied, and pointed out further (8) that Galli Valerio's note on natural infection in *lutzi* offered no serious obstacle

<sup>1</sup> The studies and observations on which this paper is based were conducted with the support and under the auspices of the Medical Services of the Crown Colony of Trinidad and Tobago, British West Indies, and the International Health Division of The Rockefeller Foundation.

to this view, as it was highly improbable that a non-expert could give a final identification to a much damaged specimen from a difficult taxonomic group.

Knab's authority was sufficient to end the discussion at that time, and for many years little or no attention was given to the idea that *Kerteszia* species might be malaria vectors. Further, the only two important papers on the subject that followed before 1937 lent no support to the possibility that bromeli-colous anophelines were involved in malaria transmission. Thus, Nelson Davis (9) concluded that although *Anopheles* (*Kerteszia*) *bellator* was present, it was not the vector in an outbreak of mountain malaria which he investigated in São Paulo. Darling (*vide* Hackett (10)) dismissed the possibility that *Anopheles* (*Kerteszia*) *neivai* H. D. & K might be important in Panama, and saved considerable waste by stopping an expensive bromeliad control project that had been contemplated without adequate preliminary investigation.

The likelihood that *A. bellator* was a vector in Trinidad had been considered very early in the century by a local entomologist, F. W. Ulrich, whose views are referred to by Sir Rupert Boyce (1). Ulrich himself did not publish his ideas, although they were fully discussed with his associates, one of whom, de Verteuil, later produced a very thorough epidemiological argument in their favor. It is possible that Ulrich was influenced by the criticisms of Knab; in his 1913 list of Trinidadian mosquitoes (11), where biological and economic notes are given, he alludes to *Anopheles tarsimaculatus* (= *aquasalis* Curry) as a vector but makes no comment on *A. bellator*.

In two later publications from Trinidad, Lassalle (12) and de Verteuil (13) also attached no importance to *bellator*, but in 1935 de Verteuil revised his position following a detailed survey of the cacao-growing areas of Cumuto, Talparo, and Tamana. In his report to the Surgeon General for that year (2) he notes that the only common ground-breeding anopheline was *A. oswaldoi*—"a non-malaria carrier and non-househaunter; whilst towards evening, between 4 and 8 p.m., large numbers of *A. bellator* adults were found to be swarming in every house and village in the district whenever they happened to adjoin a cocoa estate." He inferred "that the high malaria incidence and mortality of these districts was due to *Anopheles bellator*—." De Verteuil's epidemiological inference was fully confirmed by Rozeboom and Laird in 1942 (3). In collections of *bellator* taken from the same area that de Verteuil had studied (Cumuto) these authors found three naturally infected specimens out of the 725 they examined: two with oocyst-infected stomachs and one with sporozoite infected salivary gland. Downs, Gillette, and Shannon (4) shortly afterwards published the results of a malaria survey of the island. They, too, found conclusive evidence of *bellator*'s vector status, reporting several experimental and natural infections.

By 1941 the question of bromeliad malaria had been reopened in Brazil, and Fonseca and Correa (14) obtained experimental infection of the species Lutz had originally suspected, *A. cruzi* D. & K. Later, Correa (15) investigated a malaria outbreak among the personnel constructing the Santos-São Paulo Road in the Serra do Mar forests of São Paulo. He found that *cruzi* was the only adult present, and four specimens out of the 75 that he dissected were naturally infected,

three having oocyst infections and one a sporozoite infection. There is now good evidence that in the states of São Paulo and Paraná the problem of bromeliad malaria is a real and extensive one.

#### THE ECOLOGICAL BASIS OF BROMELIAD MALARIA IN TRINIDAD

Knab's discussion of 1912 in which he criticizes Lutz's views is of importance because it states clearly the significant facts that are associated with the transmission of insect-borne disease. He points out that "it is not sufficient that occasional specimens bite man, as is the case with forest mosquitoes. Although a person may be bitten by large numbers of such mosquitoes the chances that any of these mosquitoes survive to develop the parasite in question and then find opportunities to bite and infect another person, are altogether too remote." His position here is clearly that which was formulated so succinctly at a later date by Hackett (10), who maintained that the essential feature of any vector was that it would "bite man and bite him repeatedly."

Knab's implicit assumption was that a "forest" mosquito would never have this chance. However, it is clear from the two essential epidemiological features of bromeliad malaria in Trinidad that this is by no means necessarily so. In the first place it is seen that all forest mosquitoes are not equally restricted to forest cover: some may leave it more or less regularly. Secondly, man may create, and inhabit, artificial forests of tree crops which are highly attractive to some forest mosquitoes.

#### *The Ecology of A. bellator*<sup>2</sup>

Forest mosquitoes cannot be grouped as a single ecological category; different species may be orientated within a limited range on the climatic gradient that is presented by the vertical transition from high humidity and shade near the forest floor to the relative dryness, light, and wind of the canopy (Bates (16); this paper gives further reference). Thus it is apparent from the ecological studies of Pittendrigh (17) that *A. bellator* has well-defined limits within the range of microclimates offered by natural forests. Again, it has been shown in the case of *Haemagogus capricornii* (16) that this species occurs in the higher, less humid levels of forests and also on the ground at the edge of forest clearings. These facts apply also to *Anopheles bellator*. The daytime distribution of *bellator* within the seasonal forests<sup>3</sup> of Tamana (Trinidad) is shown in fig. 1. It is far more abundant in the immediate subcanopy region than it is on the ground, but is equally abundant in open spaces in the forest where conditions at ground level simulate those of the canopy. Although a mosquito of the forest in one sense, it may leave this environment and pass at lower levels through second growth and more open vegetation to nearby villages. On the other hand a closely related species, *Anopheles (Kerteszia) homunculus* Komp, has widely different ecological characteristics: throughout the day this species occurs in maximum numbers

<sup>2</sup> Vide (17) for a fuller discussion and experimental detail.

<sup>3</sup> Vide Beard, J. S. "Climax Vegetation in Tropical America," *Ecology*, vol. 25, no. 2, 1944, for a classification of American forest types.

near ground level within the forests where conditions are highly humid and shaded (fig. 1); in contrast to *bellator*, it leaves the forest and enters open vegetation to a far less extent. It is much more nearly the "forest" mosquito that

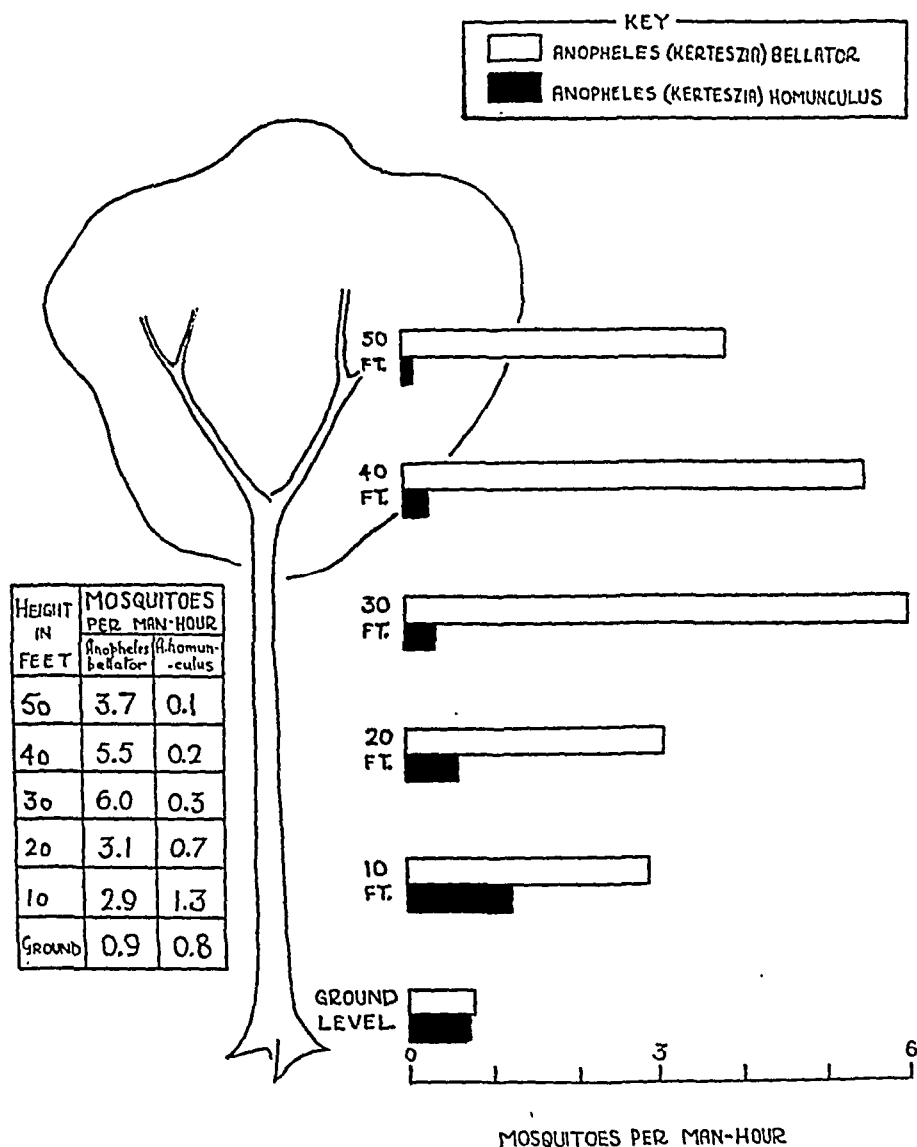


FIG. 1. THE VERTICAL DISTRIBUTION OF *ANOPHELES BELLATOR* AND *ANOPHELES HOMUNCULUS* IN THE SEASONAL FORESTS OF TAMANA, TRINIDAD, B.W.I.

Knab envisaged, and although it probably is responsible for some transmission in the very humid parts of the island it is by no means as important a vector as *A. bellator*.

#### *The Cacao Industry*

The other factor of importance in producing bromeliad malaria in Trinidad is the cacao industry. Throughout great sections of the center of the island, forest has been replaced by plantations of *Theobroma cacao* (cocoa). In the Trinidadian



estates the cacao tree itself is protected by tall, regularly interplanted shade trees, the immortelles (*Erythrina glauca* and *E. micropteryx*), and the plantations as a whole constitute an extensive artificial forest.

The first and most important feature of this situation is that, unlike any natural forest, the *bellator*-inhabited cacao forest supports a large human population whose livelihood is based on their daily labor within or near it. The industry is, in fact, primarily responsible for the contact between man and the forest mosquito.

Several special features of the cacao industry are of interest. The very wide planting of the immortal trees, twenty-five feet by twenty-five, and their deciduous habit in the dry season produce an internal climate that differs radically from adjacent natural forests. It is both lighter and drier. The humidity at ground level is often lower than it is in the immediate subcanopy of the forest.

TABLE 1

*The thirty-four species of bromeliads found on the immortal shade trees of Trinidad cacao estates*

GENERA	SPECIES
Tillandsia.....	<i>utriculata</i> , <i>flexuosa</i> , <i>fasciculata</i> , <i>bulbosa</i> , <i>sumbricata</i> , <i>rubra</i> , <i>complanata</i> , <i>stricta</i> , <i>Gardneri</i> , <i>monadelpha</i> , <i>anceps</i> , <i>usneoides</i>
Vriesia.....	<i>simplex</i> , <i>albiflora</i> , <i>procera</i> , <i>macrostachya</i> , <i>platynema</i> , <i>amazonica</i>
Guzmania.....	<i>lingulata</i> , <i>sanguinea</i> , <i>monostachia</i>
Thecophyllum.....	<i>capituligerum</i> , <i>Splitgerberi</i>
Catopsis.....	<i>Berteroniana</i> , <i>floribunda</i> , <i>sessiliflora</i>
Hohenbergia.....	<i>stellata</i>
Wittmackia.....	<i>lingulata</i>
Gravisia.....	<i>aquilega</i>
Aechmea.....	<i>Mertensii</i> , <i>porteoides</i> , <i>dichlamydea</i> (var. <i>trinitensis</i> ), <i>nudicaulis</i>

All bromeliad species in which *Anopheles* (*Kerteszia*) larvae have ever been found are in italics. The italicized species are not equally important (*vide* table 3).

Consequently the *bellator* population, although much smaller than that of the forest, occurs at lower levels; and this is clearly of epidemiological significance.

#### *The Bromeliad Flora of Immortelle Trees*

A further important consequence of the internal climate and structure of cacao estates is the flora of sun-epiphytes which develops within it. This flora occurs predominantly on the immortal trees and comprises at least eight families of flowering plants of which the Bromeliaceae are by far the most conspicuous. We have observed on immortelles 34 out of the 60 Trinidadian species; they are listed in table 1. One half of these are common, about ten are abundant, and five may be described as universal, being present on almost every tree. The density and regularity of the immortal community of epiphytes can scarcely be overemphasized. With the exception of very young replacements it can be said that every tree supports a bromeliad flora, and the number of individual plants

on most trees is astonishing. Table 2 lists counts of the bromeliads on five separate profiles from distinct estates in the Tamana region. For these 50 trees, the average number of bromeliads per tree was 66.

The immortelle community of bromeliads is fairly constant throughout the island. There are changes in the composition of the community in the climatic extremes, and differences in the relative frequency of the common species in different areas. However, the following five bromeliads are found in abundance on nearly all cacao estates: *Aechmea nudicaulis*, *Gravisia aquilega*, *Vriesia procera*,

TABLE 2

*The bromeliad flora on the immortelle shade trees of cacao estates*  
Sample enumeration strips from five different Tamana estates

BROMELIAD SPECIES	STRIP #1 NINE TREES	STRIP #2 ELEVEN TREES	STRIP #3 TEN TREES	STRIP #4 TEN TREES	STRIP #5 TEN TREES	TOTAL FOR FIFTY TREES	AVERAGE NUMBER PER TREE
<i>Aechmea nudicaulis</i> .....	108	143	172	132	155	710	14.2
<i>Gravisia aquilega</i> .....	119	152	128	109	191	699	14.0
<i>Vriesia procera</i> .....	121	107	121	214	99	662	13.2
<i>Guzmania monostachia</i> .....	87	91	157	58	211	604	12.1
<i>Tillandsia fasciculata</i> .....	31	90	24	46	39	230	4.6
<i>Wittmackia lingulata</i> .....	0	0	0	0	160	160	3.2
<i>Catopsis sessiliflora</i> .....	39	10	3	0	10	62	1.2
<i>Vriesia amazonica</i> .....	9	26	1	2	18	56	1.1
<i>Catopsis floribunda</i> .....	2	4	4	24	1	35	0.7
<i>Guzmania sanguinea</i> .....	0	0	0	26	0	26	0.5
<i>Guzmania lingulata</i> .....	0	0	0	0	25	25	0.5
<i>Vriesia macrostachya</i> .....	1	0	13	10	0	24	0.5
<i>Tillandsia anceps</i> .....	0	0	5	12	0	17	0.3
<i>Tillandsia bulbosa</i> .....	0	0	8	4	0	12	0.2
<i>Vriesia platynema</i> .....	0	0	11	0	0	11	0.2
<i>Hohenbergia stellata</i> .....	5	2	2	0	0	9	0.2
<i>Thecophyllum Splitgerberi</i> .....	0	0	1	1	0	2	0.04
<i>Catopsis Berteroniana</i> .....	0	0	0	2	0	2	0.04
<i>Vriesia albiflora</i> .....	0	0	0	1	0	1	0.02
Total: all species.....	522	625	650	641	884	3,322	66

*Guzmania monostachia*, and *Tillandsia fasciculata*. The cacao areas illustrated in fig. 2 are in many cases almost uninterrupted forest. The number of immortelles to the acre varies from 11 in abandoned areas to 64. Bearing in mind the data of table 2, which shows the density on individual trees, some impression can be gained of the great size and density of bromeliad populations—and therefore anopheline breeding grounds—in the cacao area. Cladimiro Picado's description of epiphytic bromeliad floras as "les mares aeriennes" seems very apposite in this context (18).

The extent of *bellator* breeding in this bromeliad flora is limited in two ways. In the first place, it is restricted to certain species of bromeliads and, secondly, it does not occur in the drier parts of the island.

*The Incidence of Breeding in Different Bromeliads*

Both from direct observation of the incidence of larvae in different species in the field and from the results of experiments in which comparable samples of different bromeliads were offered for oviposition in the same "breeding-plot," it is clear that the many different immortelle bromeliads are by no means equally important as host plants of *A. bellator*. There is evidence of a host plant selection on the part of the anophelines which is of practical value.

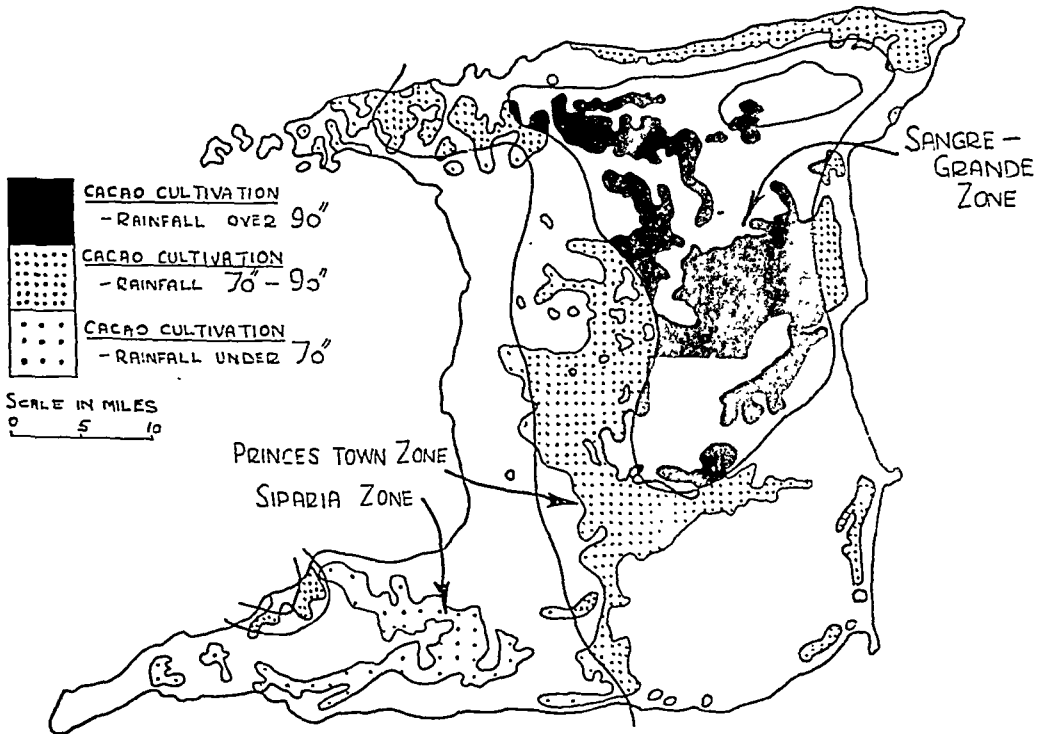


FIG. 2. THE CACAO AREAS OF TRINIDAD, B.W.I.

The shaded areas are under cacao cultivation. Intensity of shading indicates density of rainfall in the area concerned

For example, the two commonest large bromeliads on the immortalles of Trinidadian cacao estates are *Aechmea nudicaulis* and *Gravisia aquilega*. *Gravisia aquilega* (fig. 3) is by far the most important plant in the complex of *bellator* breeding in Trinidad. It is rare to find a plant of this species on cacao estates that does not have *bellator* larvae in it. On the other hand it is equally rare to find *bellator* in *Aechmea nudicaulis*. These two plants occur side by side even on the same limb of the tree, and the important difference between them always holds. Together they form nearly one-half of the total immortelle community of bromeliads. Again, *bellator* is rare in many of the other common bromeliads of the cacao estates, such as *Tillandsia fasciculata*.

Table 3 illustrates the relative importance of the common bromeliads. The frequency of the plants is based on the five profile enumerations given in table 2; the frequency of *bellator* larvae is based on the results of an experimental breeding



FIG. 3. *GRAVISIA AQUILEGA* (SALISB.) MEZ.

- (a) (Upper) growing on an immortal shade tree (*Erythrina micropteryx*) in a cacao estate, Penal, Trinidad, B.W.I.  
(b) (Lower) Heavy infestation of a roadside tree.

plot in which equal samples of the species were offered for oviposition. The figure obtained by multiplying these two frequencies is used here simply as a more valuable indication of the practical significance of different bromeliads than is either of the frequencies considered separately.

Pittendrigh (17) considers two groups of factors in discussing the relative importance of different bromeliad species as host plants of the anophelines. Firstly, there are important simple physical differences between bromeliads. Some species hold little or no water at all, and others hold only small amounts immediately after rain. Clearly such bromeliads play no part in bromeliad-anopheline breeding. Among true "tank" species of bromeliads which regularly hold water, it is shown that there is a general relation between the plant's water holding capacity and the extent of its use by the mosquitoes. Finally, under this head-

TABLE 3

*The relative importance of ten common bromeliads as host plants of Anopheles bellator*

BROMELIAD SPECIES	BROMELIAD FREQUENCY PER TREE*	A. BELLATOR LARVAE PER PLANT†	A. BELLATOR LARVAE PER SPECIES PER TREE
<i>Aechmea nudicaulis</i> .....	14.2	0.02	0.3
<i>Gravisia aquilega</i> .....	14.0	1.8	25.2
<i>Vriesia procera</i> .....	13.2	0.2	2.6
<i>Guzmania monostachia</i> .....	12.1	0.04	0.5
<i>Tillandsia fasciculata</i> .....	4.6	0.0	0.0
<i>Wittmackia lingulata</i> .....	3.2	1.1	3.5
<i>Catopsis sessiliflora</i> .....	1.2	0.0	0.0
<i>Vriesia amazonica</i> .....	1.1	4.8	5.3
<i>Catopsis floribunda</i> .....	0.7	0.0	0.0
<i>Hohenbergia stellata</i> .....	0.2	1.3	0.3

\* Vide table 2.

† These figures were obtained from an experimental breeding plot on the ground in which equal samples of all ten plants were offered for oviposition (*vide* (18) for detail).

ing it is noted that the relative inaccessibility of the water surface probably plays a major part in rendering unimportant such species as *Aechmea nudicaulis*, which has a high, narrow, tubular rosette of leaves that are stiffly erect above the inter-foliar water.

Secondly, there are purely ecological factors involved. It is suggested that the ecological divergence of *A. bellator* and *A. homunculus* into different ranges on the vertical gradient of climate within the forest brings the two mosquitoes into contact with substantially different bromeliad floras, since these are also stratified (17). This has two consequences. Evidently the fine distribution of the anophelines in nature may not coincide with that of some plant species that would otherwise constitute most acceptable breeding grounds, as is seen from the results of breeding experiments when they are artificially offered for oviposition. A further consequence is apparently the (ecobiotic) fact that in such breeding

experiments *Anopheles homunculus*, from the lower forest levels, is more willing than is *A. bellator* to utilise the small plants with a poor water content that predominate in lower forest strata.

### *The Geographic Distribution of A. bellator in Trinidad*

Although *bellator* occurs in the drier sections of the microclimatic gradient of the wet forests in the center of the island, there is nevertheless a limit to its toleration of low humidity. Pittendrigh (17) shows that the density of *bellator* falls off at elevations higher than the optimum which is in the subcanopy, and that the mosquito moves down the profile on drier days and in general in the dry season.

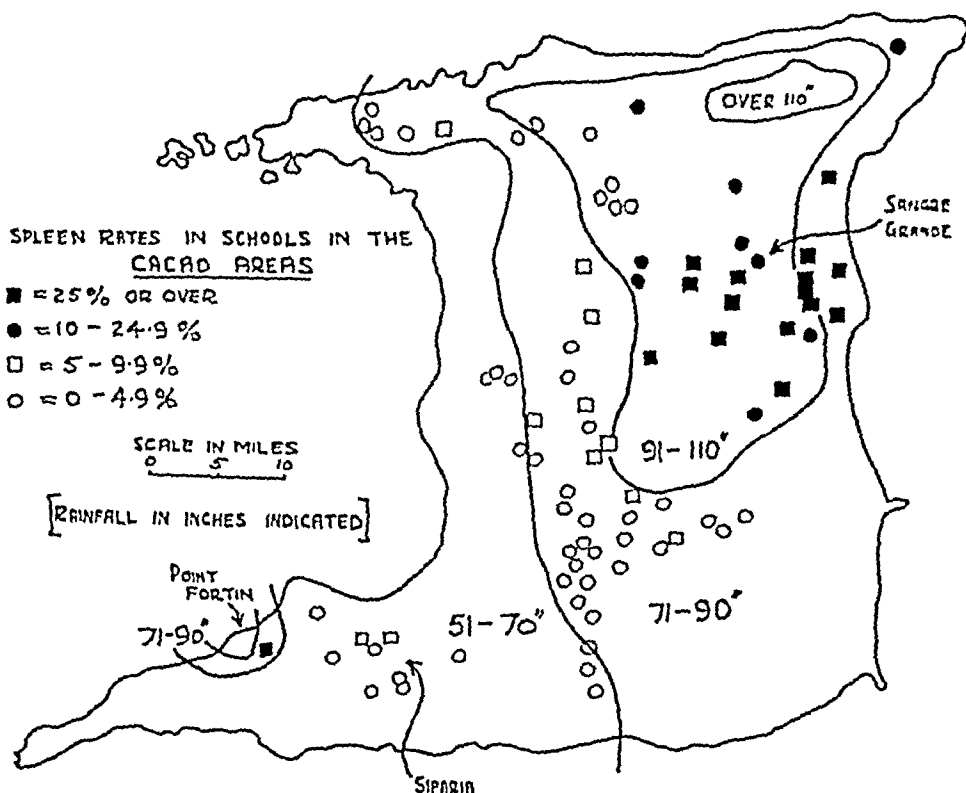


FIG. 4. SPLEEN RATES IN THE CACAO AREA  
Note the correlation of high rates with high rainfall

He correlates with this the geographic distribution of the mosquito in the island. Downs, Gillette, and Shannon (4) have already noted that the distribution of high spleen rates in the bromeliad malaria zone is correlated with high rainfall.

Fig. 4 shows the distribution of spleen rates in all schools in the cacao-growing areas. The schools represented include all those where the malaria concerned is *bellator* transmitted: there are no localities with *bellator* malaria that are not in the cacao area.

It is seen from the map that the heavy rates are all restricted to one well-marked zone with a rainfall of 90-110 inches around the town of Sangre Grande in the northcentral part of the island. The only vector present is *A. bellator*.

Most of the remaining schools in the cacao area have much lower spleen rates, especially toward the South and West associated with the Siparia and Princes Town zones of cacao (fig. 2).

The bromeliad floras on the cacao estates here (Princes Town and Siparia) are almost as heavy as in the Northeast, near Sangre Grande. Relatively more of the smaller xerophytic bromeliads are present than in the Sangre Grande cacao, but the difference is not great. However, *bellator* is absent or extremely rare. The rainfall in this area is low and there can be no doubt that the density of *bellator* populations is correlated with this factor. What little malaria there is in the Princes Town and Siparia cacao zones is due to the dry season immigrations of *Anopheles aquasalis* upstream from the coastal areas.

Table 4 illustrates the change in the density of *bellator* populations moving from the zone of heavy precipitation in the Northeast (Cumuto) through the intermediate stations Muendo Nuevo and Busy Corner to the zone of low precipitation in the Southwest (Siparia).

TABLE 4  
*The relation between rainfall and the density of Anopheles bellator*

MARCH, 1942		PLANTS EXAMINED FROM IMMORTELE TREES ON CACAO ESTATES					
		<i>Gravisia aquilega</i>			<i>Wittmackia lingulata</i>		
Name of locality	Rainfall zone	Number of plants	Total larvae of <i>bellator</i>	<i>bellator</i> larvae per plant	Number of plants	Total larvae of <i>bellator</i>	<i>bellator</i> larvae per plant
Cumuto.....	91-100"	33	112	3.3	28	19	0.7
Mundo Nuevo.....	81-90"	20	30	1.5	18	8	0.4
Busy Corner.....	71-80"	19	6	0.3	15	1	0.06
Siparia.....	61-70"	43	0	0.0	44	0	0.0

A small area around La Brea and Point Fortin in the southwest peninsula provides a further illustration of the point. From fig. 2 it can be seen that the rainfall in this small area is considerably higher than that of all the surrounding Siparia zone where *bellator* is absent. In this "oasis" of higher precipitation in the La Brea area, *bellator* reappears and the spleen rates are correspondingly higher.

#### THE CONTROL OF BROMELIAD MALARIA

##### *Note on the Behavior of A. bellator*

The behavior of *Anopheles bellator* precludes any attempt to control it by anti-adult measures, such as spray killing, which have recently been advocated for certain domestic species. Although it does on occasion enter dwellings to feed—and often in numbers—it never stays indoors, but leaves for forest cover immediately after feeding. De Verteuil (2) has already noted this and remarks that "it is quite exceptional to find them on the walls or on the mosquito nets . . . ." We have never observed *bellator* resting on house walls at any time. It has been

seen on stable walls after feeding, but even here its rest is not long, and within an hour after the peak of the main evening flight there are none left. Again, although *bellator* has a well-marked evening flight, it is active throughout the day, especially in forests and cacao plantations.

The greater part of transmission must take place either in the cacao field, where laborers are freely attacked on damp days (fig. 5), or on the veranda of houses at evening, where it is the custom of country people in Trinidad to gather and cook the evening meal. The flight actually inside bedrooms is almost



FIG. 5. LABORERS SITTING DOWN TO WORK IN A CACAO ESTATE  
On damp days they are heavily bitten by *Anopheles bellator* and *Anopheles homunculus*

TABLE 5

*House captures of anopheles bellator and anopheles homunculus made with human bait simultaneously outside, on the veranda, and inside the bedroom*

24 NIGHTS BETWEEN JAN 30 AND MAR 31, 1945	ANOPHELES BELLATOR				ANOPHELES HOMUNCULUS			
	Outside	Veranda	Bedroom	Total	Outside	Veranda	Bedroom	Total
Total	229	140	2	371	44	15	1	60
Average per night: 6-7.30 p m.	9.5	5 8	0.08	15 4	1 8	0 6	0 04	2 5

negligible, but the flight on the veranda is regular and heavy (table 5). The only possible approach to controlling *bellator* malaria is apparently the prodigious task of controlling the anophelines breeding grounds, the epiphytic bromeliad flora of cacao estates.

*The Manual Removal of Bromeliads*

The most obvious approach to the problem of destroying bromeliad populations is to remove the plants by hand, and this method has been employed in



Trinidad in a few special instances connected with the war emergency. It has, however, never been possible to adopt the method as a regular measure to meet the real problem, which is that confronting the agricultural community in the thinly populated cacao areas of the island. The principal objection to hand removal of bromeliads as a control technique is its extremely high cost, which is an inevitable consequence of the sheer immensity of bromeliad populations on *immortelles*. The present rate of pay demanded by those laborers who are willing to climb the *immortelles* varies from BWI \$1.50 to BWI \$2.00 per tree. The number of trees to the acre varies from 11 to 64, so that the costs per acre can be cited as from \$16.50 to \$96.00 (\$1.50 per tree), or \$22.00 to \$128.00 (\$2.00 per tree).

In most cases the local communities in the cacao areas are not aggregated into well-defined villages. The population as a whole is scattered, and no house is far from the great stretches of cacao "forest." Indeed most of the dwellings of the laborers and small holders are situated well within the estate itself. This necessitates the clearing of large areas to protect relatively few people; and with the cost of control as high as that cited above, the work cannot be undertaken.

This cost can, however, be very substantially reduced by the adoption of a "species control" policy based on the fact that the larval population of *bellator* is restricted to certain bromeliad species. In Trinidad such a policy would be best carried out by removing only *Gravisia aquilega* and the two less common species resembling it, *Wittmackia lingulata* and *Hohenbergia stellata*. It would involve leaving the very common species, *Aechmea mudicaulis*, and the many *Tillandsia* and *Guzmania* species.

Even with costs adequately lowered by such a "species control" policy, it is still doubtful whether removal of bromeliads by hand could ever be adopted as a regular control technique, for several additional difficulties attach to the use of this method in the particular circumstances obtaining in Trinidad.

The *immortelle* trees which account for almost the entire problem are singularly difficult and dangerous to climb. The difficulty in climbing is due in the first place to large thorns (piquants) with which the tree is covered. These thorns always cause bad scratches and even open lacerations among men working on the trees for any length of time. Moreover, the task is made more objectionable by the scorpions, spiders, and snakes that are harbored by the luxuriant growth of miscellaneous epiphytes. Among field assistants employed in routine investigational work on bromeliads, scorpion and spider bites are common, and serious encounters with snakes have occurred several times. The snakes usually present among the epiphytes on *immortelles* are not venomous species, but under the shock of the encounter most men will become panic stricken and jump from the tree.

A more common and equally serious danger involved in climbing the *immortelle* is the tendency of the limbs of this tree to break off easily. The wood is extremely brittle and the limbs are long, tenuous, and often diseased without this being apparent from the outside. In the course of one bromeliad-removal program which lasted a month, three serious injuries, including one fatality, occurred among the laborers employed.

Lastly, consideration has to be given to the fact that climbing is almost impossible when the tree is wet, owing to the insecurity of the climber's grip and especially to the increased chance of branches breaking when they are further burdened with the immense weight of water held by the bromeliads after rain. This apparently simple point becomes of real significance when it is realized that the great bulk of control work is required in the very wet parts of the island where it may rain, at least part of the day, for days and often weeks on end. Under these circumstances not only the continuity of the work but the maintenance of trained crews becomes impossible. The maintenance of well-trained labor gangs would be essential for species control work on the bromeliads.

### *The Spray Killing of Bromeliads<sup>4</sup>*

In looking for an alternative method of destroying extensive *bellator* breeding grounds, an attempt has been made to develop a technique of spray killing the bromeliads. The principal attraction of any possible spraying method is that it will obviate the main objections to removal of bromeliads by hand, viz., the danger to operators, the high expense due to the slowness of the process, the inevitable discontinuity of work, and the consequent inability to offer regular employment for trained personnel.

There is, however, one considerable problem connected with the development of a spray killing method: this is the necessity for a strict selective action on the part of the herbicide to be used in destroying the bromeliads. Any herbicidal spray that is used for this purpose on cacao estates will cover the commercially valuable cacao trees and their immortelle shade and therefore must be innocuous to them. Herbicides such as sodium arsenite could therefore not be used.

The possibility of developing a selective bromelical spray arises from the remarkable physiological properties of bromeliad leaves, which are capable of absorbing not only water but organic acids and mineral salts. Epiphytic bromeliads, barred from access to the soil, are dependant for their water and nutrient supply upon the tank of the interfoliar water and its content of organic detritus. The root system of these plants is almost entirely mechanical in its function, serving to attach the plant to the tree. The intake of water from the tank is carried out by the leaves, which are equipped with a system of very elaborate epidermal absorptive organs, or "trichompompe," as Mez (20) has called them. The ordinary plant leaf has, of course, no such regular function; its cuticularised epidermis is practically impermeable to water and salts.

This difference between bromeliad leaves on the one hand, and ordinary leaves such as cacao and immortelle on the other, has made it possible to use several chemical sprays as efficient selective bromelicides in cacao estates. Of these sprays copper sulphate has so far given the best results.

Fig. 6 shows a limb of an immortelle tree supporting five plants of *Gravisia aquilega* killed with a spray of 2 per cent copper sulphate solution. The whole of this immortelle was covered by the spray but has not been affected by it. In

<sup>4</sup> *Vide* (19) for a fuller account.

fig. 7 another specimen of *Gravisia aquilega* is seen. This plant was killed with a lead arsenate spray.

Several engineering difficulties are encountered in using the spraying method on a large scale in the field. It is necessary to employ spraying equipment that will deliver the spray to a height of 70 or even 100 feet above ground to ensure



FIG. 6. *GRAVISIA AQUILEGA*. FIVE DEAD PLANTS ON A BRANCH OF AN IMMORTELE TREE

These plants were sprayed with a 2 per cent solution of copper sulphate

that none of the plants will be out of range of the method. Equipment capable of doing this is bulky and very heavy, and cannot be used in the interior of cacao estates.

The spraying equipment developed by the United States Department of Agriculture for use in the control of the gypsy moth (*Lymantria dispar*) in the New England forests has been selected as that which is best adapted to the particular demands of cacao estate conditions and the restrictions imposed by their com-

mercial importance. The essential advantage of this method is that the equipment is not taken into the estate itself. The power developed by the pumping unit is sufficiently great to allow it to be left outside the areas of treatment, and the whole unit is mounted on a truck based at a convenient roadside water supply from which the hose lines are extended into the estates for spray operations. Only the hose is carried into the interior of the estate, and in this way the diffi-



FIG. 7. *GRAVISIA AQUILEGA* ON AN IMMORTELLE TREE  
This plant was killed with a dilute lead arsenate spray

culties of terrain and the necessity to avoid mechanical damage to closely planted trees are overcome.

One large control project has been carried out in Trinidad using this technique and the above-mentioned equipment. In terms of cost, speed, and safety it proved much superior to the earlier programs in which bromeliads were removed by hand, and it holds out good promise that the method, when fully developed, will be a satisfactory and economical procedure for the rural areas of central Trinidad.

It is believed that it will not be necessary to re-treat thoroughly sprayed areas for several years, possibly five or more, in view of the facts that any new infestation will have to start from seed and that the growth of the seedlings of large bromeliad species is very slow.

### *Changed Agricultural Practice*

It has already been emphasized in this paper that the focal point of the bromeliad malaria complex in Trinidad is the particular form of cacao cultivation that is adopted. The problem is man made. The presence of regularly interplanted immortal trees in the estates renders them forests of high light intensity and low humidity that support great *bellator* breeding grounds and an adult population of this mosquito at lower levels than that in the forest. It is therefore of great interest that the use of immortelles is far from universal in the many countries where the crop is cultivated. Elsewhere other trees are employed for shade, and in more than one country cacao is grown with great success in the complete absence of "interplanted shade."

This is the case in the Gold Coast and particularly in Grenada, B.W.I. Where interplanted shade is dispensed with, the cacao tree, in nature an understory species, is afforded protection by rows of trees placed at varying distances perpendicular to the prevailing wind. Were it adopted in Trinidad, this system of windbreaks would have a great effect upon the malaria conditions of the central part of the island. The immortal can apparently not be bettered as interplanted shade, so that there is little likelihood that a tree less disposed to develop bromeliad infestations could be found as a substitute. But were the system entirely dispensed with in favour of windbreaks, it would be easy to select one of several trees such as the mango, that would have the advantage of commercial utility (which the immortal does not have) added to the attraction that it would not develop the great bromeliad flora of the immortal.

The use of windbreaks would be of greater value in a further way. They would not afford the conditions of continuous artificial forest afforded by interplanted shade, and thus, quite apart from any consideration of the bromeliads, the adult *bellator* population would be reduced or eliminated.

In 1944 the Government of Trinidad and Tobago introduced a new cocoa subsidy (totaling BWI \$4,000,000), which, it is hoped, will assist the industry to recover from the depression it has suffered in the past two decades. By means of this subsidy the Department of Agriculture is, for the first time, actively encouraging the adoption of windbreaks as distinct from interplanted immortal shade, since the conditions of payment of the subsidy are such that a greater sum per acre is offered to assist those owners who replant their estates without using immortelles.

It should be stressed that although it is probable that spray killing of the bromeliads will prove an effective technique for a direct approach to controlling the present malaria situation, its value would never compare with the change in agricultural practice that is contemplated here. Following the widespread adoption of windbreaks the endemic bromeliad malaria of the cacao areas could be

expected virtually to disappear, for such a change would constitute the removal of the ecological basis on which the disease rests at present.

The most thorough spray killing would leave untouched the fundamental cause of the present endemicity of bromeliad malaria in Trinidad, namely, the ecological structure of existing cacao estates, the natural consequence of which is a heavy bromeliad and bromeliad-anopheline population in immediate contact with a settled human community.

#### SUMMARY

Lutz (1903) first considered the possibility that anopheline mosquitoes of the subgenus *Kerteszia* might be involved in malaria transmission. He concluded, on epidemiological grounds, that *Anopheles lutzi* Theobald (= *cruzi* D. & K.) was a vector in São Paulo. However, Knab, stressing the connection between the feeding habits of insects and their importance as vectors of disease strongly opposed Lutz's view, maintaining that since *Anopheles lutzi* was a forest mosquito it would not have sufficient contact with man to transmit malaria.

Independently, Ulrich and later de Verteuil believed that *Anopheles (Kerteszia) bellator* D. & K. was involved in the malaria complex in Trinidad, and this was confirmed eventually by Rozeboom and Laird, and also by Downs, Gillette, and Shannon.

It is shown that in spite of the fact that *Anopheles bellator* is a forest mosquito it is intimately associated with man in the cacao areas of Trinidad. Two principal factors are adduced to account for this contact: in the first place *Anopheles bellator* inhabits the drier microclimates of the forest and is therefore able to leave forest as such for the drier conditions offered by cacao estates and the open spaces in the villages; secondly, the cacao industry has brought relatively large human settlements into immediate contact with the forests of cacao and immortal trees, which support both larval and adult populations of *A. bellator* and heavy growths of water-holding bromeliads in which the anophelines breed.

The breeding of *A. bellator* is restricted to certain bromeliad species. Its absence in some very common species such as *Aechmea nudicaulis* is a fact of practical importance. Again, *bellator* does not occur all over the island where forests and cacao estates exist. It is restricted to areas of high rainfall.

In discussing control problems the writers do not consider that the simple removal of plants by hand could ever be adopted as a regular control procedure. It is too slow, expensive, and dangerous.

It is shown that copper sulphate may be used as a selective herbicidal spray to destroy the bromeliad infestations without damaging the commercially important cacao trees or the immortelles. In field practice the heavy spraying equipment developed by the United States Department of Agriculture for the control of the gypsy moth is found to be highly satisfactory. This control method is much faster, cheaper, and safer than removal of bromeliads by hand.

Finally it is pointed out that a change in agricultural practice, namely, the abandonment of interplanted immortal shade trees and the adoption of wind-breaks, would remove the ecological basis of the disease, which is the microcli-

matic condition produced by immortal trees in existing cacao estates. Following such a change, which is now being encouraged by the Government of Trinidad and Tobago, endemic bromeliad malaria could be expected to disappear from the center of the island.

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To Dr. V. L. Ferguson, acting director of medical services in Trinidad during 1941 and 1942, we owe an especial debt for his deep interest and ready assistance. Both Dr. MacLennan, director of medical services in 1943 and 1944 and Dr. E. J. Sankeralli, deputy director, have similarly extended great cooperation and interest.

We wish to make special mention of the hospitality and interest that was extended to us by Dr. F. C. Bishopp and Mr. R. A. Sheals of the Bureau of Entomology in the United States Department of Agriculture while we were searching for suitable spraying equipment in the United States in 1943. Without their help we could certainly not have carried the use of copper sulphate as a bromelicide to a successful and practical stage.

Dr. Lyman B. Smith of the Gray Herbarium of Harvard University has willingly advised us in taxonomic work on the bromeliads.

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The studies which are summarized in this paper could not have been carried out without the devotion of the staff of the Tamana Field Station throughout the past three years. We wish to mention particularly Mr. R. Ceden and Mr. D. Gonzales, field assistants.

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# RESULTS IN AN INFANTRY REGIMENT OF SEVERAL PLANS OF TREATMENT FOR VIVAX MALARIA

W. G. DOWNS<sup>1</sup>

## INTRODUCTION

The combat experience of this infantry regiment and its subsequent experience with malaria affords an excellent opportunity for a statistical study of vivax malaria in troops. These analyses of accumulated data were possible only because of the painstaking and prolonged planning and execution of schedules of therapy and hospitalization by Army and Navy medical officers and the careful and patient collection of data. Grateful acknowledgment is therefore made to these officers for their efforts. The official unpublished reports of Lieutenant Commander W. G. Reddick (MC), USNR, have been drawn on fully in outlining the background for these analyses which would have been impossible without these data.

The details of the experience of this infantry regiment while on a malarious island (Guadalcanal) and while undergoing an attempted "demalarialization" on a nonmalarious island (Samoa) are summarized in the following paragraphs.

The First Battalion, Regimental Headquarters Company, and special units of this regiment disembarked on a highly malarious South Pacific island on 4 November 1942 and the Third Battalion on 29 November 1942. (The Second Battalion has been excluded from this study.) The First Battalion and special units were in this malarious area for 189 days and the Third Battalion for 164 days, and both groups were in combat for 21 days. The First Battalion used quinine suppressive therapy during November 1942. With the arrival of the Third Battalion on 29 November 1942 atabrine was used as suppressive therapy, 0.4 gm. weekly (one-half tablet on week days and a full tablet on Sundays). Suppressive therapy was not rigidly enforced throughout the whole regiment until March 1943, when the roster system was begun. The regiment moved from the malarious base late in May 1943 to a nonmalarious (tropical) island and a program for "demalarialization" of the regiment was begun on 24 May 1943. On 26 November 1943 suppressive atabrine was reinstituted in the regiment.

The morning sick reports while on the malarious island showed malaria in 48 per cent of the regiment and the monthly rates (laboratory confirmed and clinical) of malaria during this period ranged roughly from 800 to 1500 per 1000 per annum. Most of the diagnoses were clinical.

For the "demalarialization" program in the nonendemic area hospital and laboratory facilities were obtained and different groups in the regiment were put on various trial schedules. No patients were treated in the hospital except on report of a positive blood smear (thick drop). Each attack was treated as it developed. Treatment of attack was uniform except in a few instances where condition of the patient necessitated a change in type of treat-

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ment. Quinine sulfate or hydrochloride (in tablet or capsule) gr. X (0.667 gm.) was given three times a day for three successive days, beginning concurrently with atabrine, gr.  $1\frac{1}{2}$  (0.1 gm.) three times a day for seven days. At the end of this week of treatment the patient was discharged from hospital to quarters and given no treatment for two days. During the next five days plasmochin gr.  $\frac{1}{6}$  (0.01 gm.) twice daily was administered. (After October 1 plasmochin was not routinely given.)

The following study is concerned with the experiences of four groups on different treatment schedules and the protocol of these groups is shown in table 1.

TABLE 1  
*Protocol of treatment groups*

GROUPS	TREATMENT PLAN	DATE MASS TREATMENT STARTED	DATE MASS TREATMENT ENDED
Group I Original strength 393	Stop all antimalarial therapy. Treat cases as they occur	Mass treatment not started	Suppressive therapy stopped 23 May 1943
Group II Original strength 690	Atabrine gr. $1\frac{1}{2}$ (0.1 gm.) t.i.d. for 7 days. Rest 10 days, repeat atabrine, 7 days, as above	24 May 1943	17 June 1943
Group III Original strength 456	No antimalarial therapy for 10 days. Then atabrine $1\frac{1}{2}$ gr. (0.1 gm.) t.i.d. 7 days. Rest 10 days, repeat atabrine as above	2 June 1943	26 June 1943
Group IV Original strength 457	Atabrine gr. $1\frac{1}{2}$ (0.1 gm.) t.i.d. for 7 days. Rest 2 days. Plasmochin $\frac{1}{6}$ gr. (0.01 gm.) b.i.d. for 5 days. Rest 10 days repeat atabrine 7 days, rest 2 days, plasmochin 5 days, as above	24 May 1943	5 July 1943

In the entire study only fifteen cases of malaria due to *Plasmodium falciparum* were noted, either singly or as part of a mixed infection. All other patients had vivax malaria. This study is concerned solely with malaria caused by *P. vivax*. From experience with other troops on the malarious island where infection was contracted, it is probable that many early cases there were caused by *P. falciparum* and that *P. vivax* cases appeared later during the study period when laboratory facilities were available for species diagnosis.

#### BASIC EXPERIENCE IN THE DIFFERENT GROUPS

Figure 1 (Groups I, II, III, IV) and the charts in Appendix I present the basic experience in the different groups.

Evacuations occurred in all four groups for various causes. These evacuation

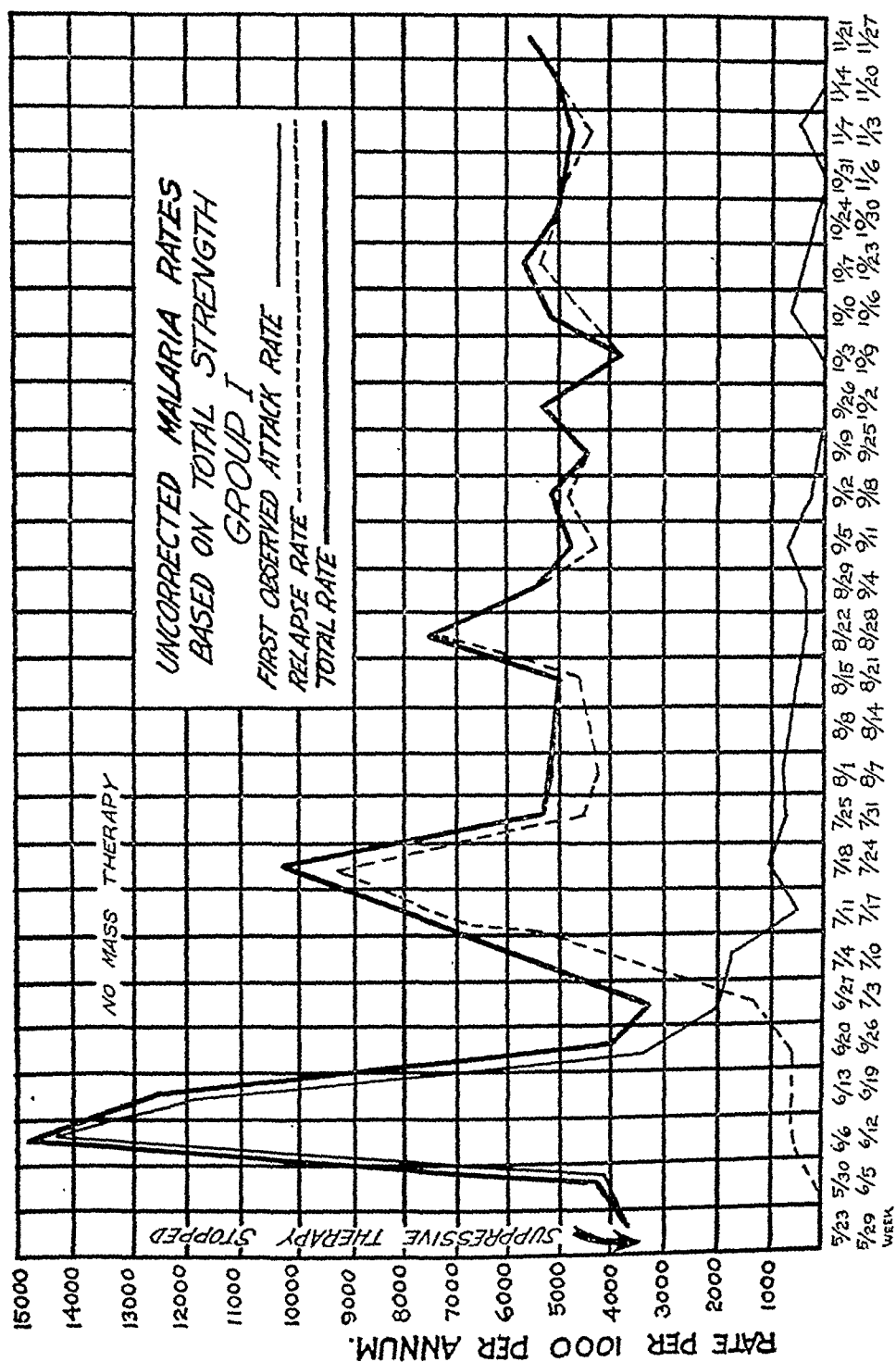


FIG. 1. WEEKLY RATES IN DIFFERENT GROUPS EXPRESSED AS RATE PER 1000 PER ANNUM Based on total strength of groups

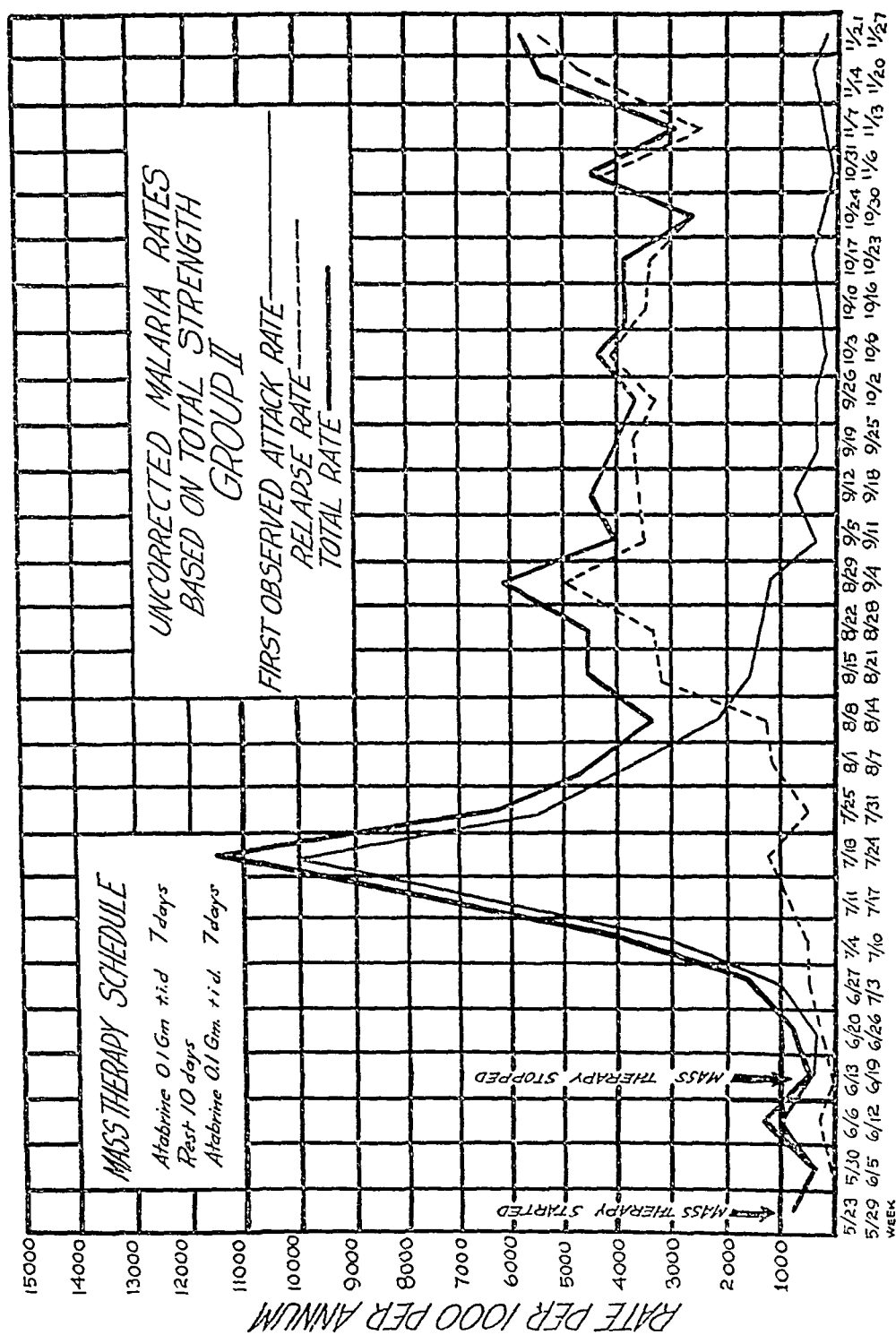


Fig. 1 (Group II)

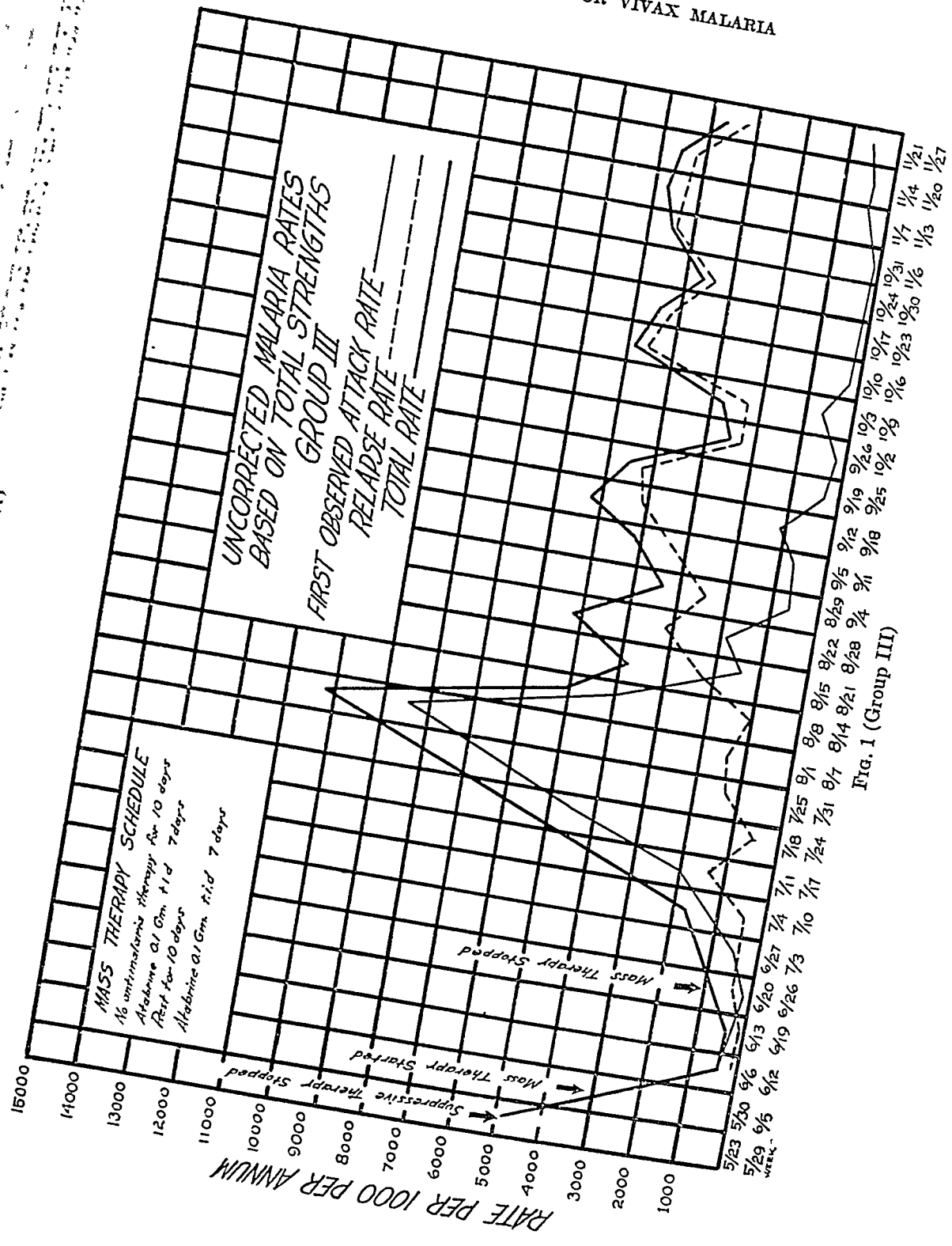


Fig. 1 (Group III)

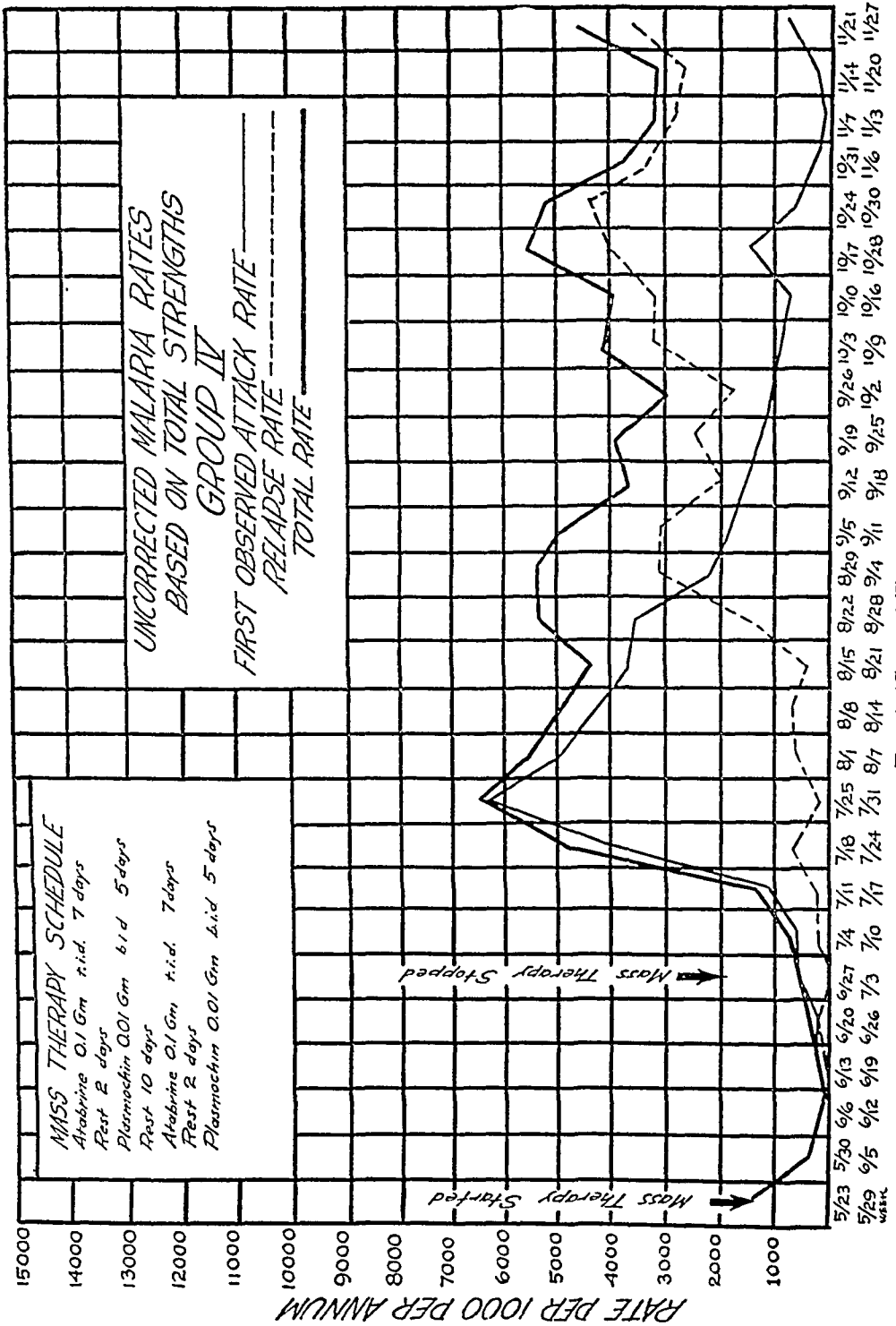


Fig. 1 (Group IV)

figures are not weighted appreciably as far as malaria is concerned, since the personnel of all groups were almost all malarious, whether evacuated for malaria or for other causes, or remaining in the original groups. Rates per 1000 per annum based on total strengths of groups have been computed weekly for all groups and shown in figure 1. From a study of these rates it is apparent that in Group I a much higher rate was reached early and total hospitalization was greater. Certain facts are not brought out clearly by the study of this figure, so further analyses are made to illustrate other significant points.

Complete records of each case are on hand, giving date of each attack of malaria and type of infection encountered, and by analysis of these records it is possible to obtain information on several points of importance.

#### I. EFFECT OF MASS THERAPY ON FIRST OBSERVED ATTACKS

Groups II and IV were selected for comparison with Group I to study this point. Group III is not included here for comparison, not because its overall experience differs markedly from the other groups but because the initial ten-day period without suppressive treatment before mass therapy was started allowed many patients to have an attack of malaria before mass therapy started and obscures a clear-cut analysis of later experience. All individuals from each group who were on hand from the period when suppressive or mass therapy was discontinued up until November 26 are entered into the analysis. Those evacuated before November 26 who had not had malaria are subtracted from strength of available individuals remaining for attacks as of the date of their departure. Comparisons are made in respect to initial attack rates and final total experience of each group.

First observed attacks refer to the first attack after discontinuing suppressive or mass therapy. The first attack thus recorded need not necessarily be the primary attack (see Section IV), since many of the individuals had had previous attacks of malaria while on the malarious island.

The attack rates are computed for the number of individuals remaining in each group available for attack, expressed as rate per 1000 per annum. As soon as an individual had his first attack of malaria (under observation) he was withdrawn from the group available for first attacks. This is necessary because in the different groups the initial experience differs greatly. In Group I, with most of the personnel subject to first observed attacks early, few individuals were left who were available for such attacks later. Fewer first observed attacks occurred later in Group I than in the other groups, but the rate based on individuals available for attack is actually as high as in the other groups (see fig. 2).

Referring to figure 2 (based on figures in Appendix II) it is evident that a high rate of first observed attacks was reached early in Groups I and II. In Group IV this peak has been flattened out considerably. However, after the initial peak in Group IV, the later rates are higher. At the termination of the period of observation for each group the rates are roughly comparable and are still high. Such rates operating on the small number of available individuals remaining in each group for a period of several months more would reduce the group not

coming down with malaria to a very small size. Table 2 indicates the experience of each group by the end of the period of observation.

Since the number of cases remaining in each of the groups is so small, and since, referring to figure 2, the rates operating on these remaining individuals are high enough to convince us that the groups would in a matter of several months be reduced almost to zero, we can make several definite statements:

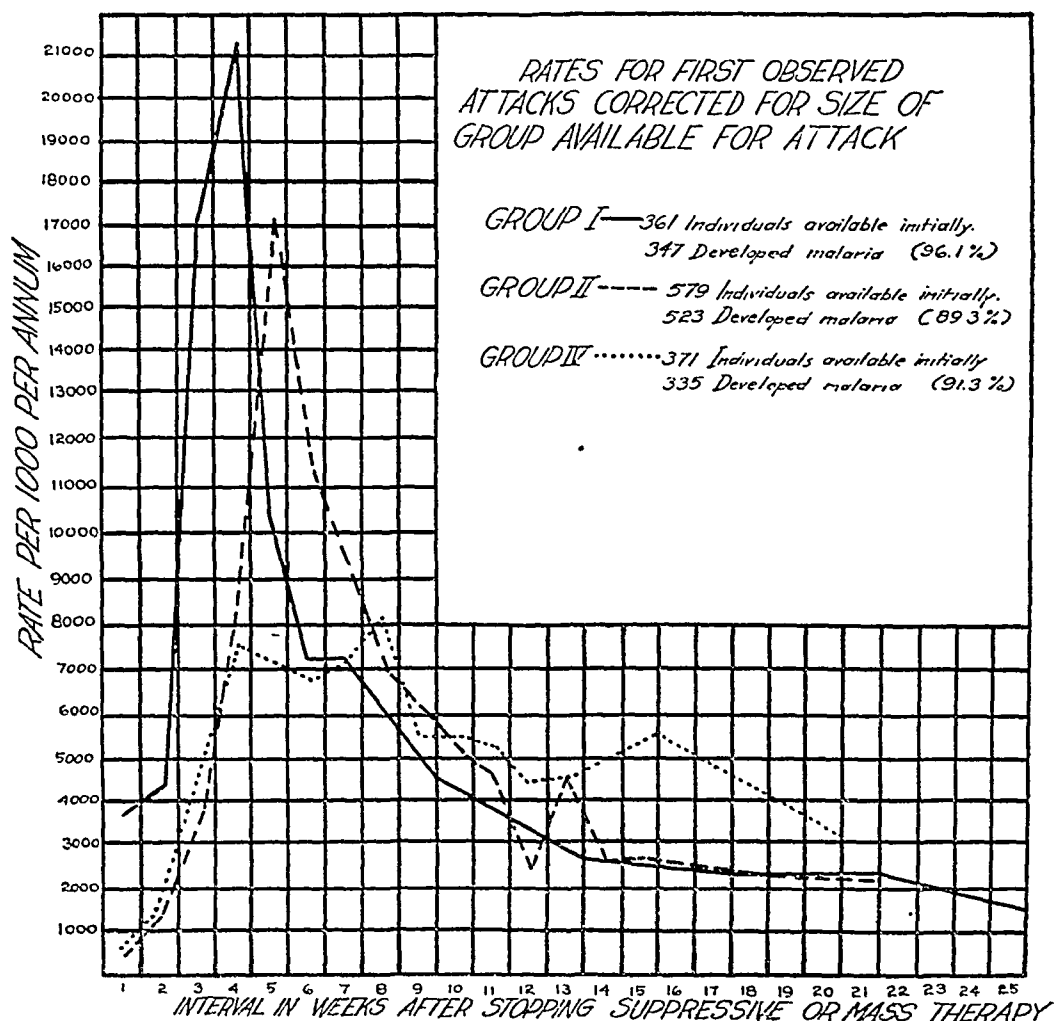


FIG. 2. WEEKLY CORRECTED RATES OF FIRST OBSERVED ATTACKS IN DIFFERENT GROUPS EXPRESSED AS RATE PER 1000 PER ANNUM  
Based on number of individuals available for such attacks

1. Suppressive therapy (atabrine) administered during a six months period of exposure apparently cured few or no cases of vivax malaria.

2. Mass therapy, either of atabrine or atabrine plus plasmochin, following after suppressive therapy (atabrine) cured few or no cases of vivax malaria.

3. Mass therapy, particularly that of atabrine plus plasmochin, alters the curve of the rate for initial attacks considerably. In Group I, which merely discontinued suppression, the highest initial attack rate and peak of cases occurred



four weeks after stopping suppressive therapy. In Group II, which had atabrine mass therapy, the peak of initial attack rate was lower than in Group I and occurred a week later. The curves after that agree very closely. Therapeutic dosages of atabrine (0.1 gm. t.i.d.) in Group II result in much higher blood levels of atabrine than does suppressive therapy of 0.4 gm. of atabrine per week in Group I. Consequently after cessation of atabrine in Group II a longer period of time is required for the blood atabrine level to drop low enough to permit vivax cases to break through. Facilities were not available to verify this with atabrine blood level studies. In Group IV, however, the initial peak of rates and of cases is remarkably flattened, although later rates remain higher. Thus the effect of this type of mass treatment has been to eliminate the peak in rate and to spread it out over a longer period of time. Whether this is a specific effect of plasmochin cannot be determined.

TABLE 2

*Incidence of first observed attacks of vivax malaria in different groups after stopping suppressive or mass therapy*

GROUP	PERIOD OF OBSERVATION	NO. OF INDI- VIDUALS INITIALLY AVAILABLE	NO. OF INDI- VIDUALS GETTING MALARIA	PER CENT GETTING MALARIA	REMAINING AVAILABLE INDIVIDUALS
	<i>weeks</i>				
I	27	361	347	96.1	14
II	23	579	523	90.3	56
IV	21	371	335	91.3	36

4. It has been noted that in all of the groups studied first observed attacks of vivax malaria (in many instances determined by history to be primary attacks) occurred as late as six months after all suppressive or mass therapy had been discontinued. Study of figure 2, showing high rates for first observed attacks still operating at the close of the study, indicates that such attacks might still be expected after an even longer interval.

## II. EFFECT OF MASS THERAPY ON RELAPSE RATES

For this study Groups I, II, III and IV are compared. The study can be conveniently divided into two parts, (1) effects on first relapses occurring after first observed attacks, and (2) effects on total relapses.

Originally, corrected rates of first relapses and of total relapses were computed for each group. This was an involved statistical maneuver and the final results did not present the picture as clearly as figure 1 does, and added no further information.

### 1. *Effects on first relapses occurring after first observed attacks.*

First relapses are not separated from total relapses on figure 1, but the first peak occurring in the relapse curve five to seven weeks after the peak of first observed attacks is largely caused by first relapses.

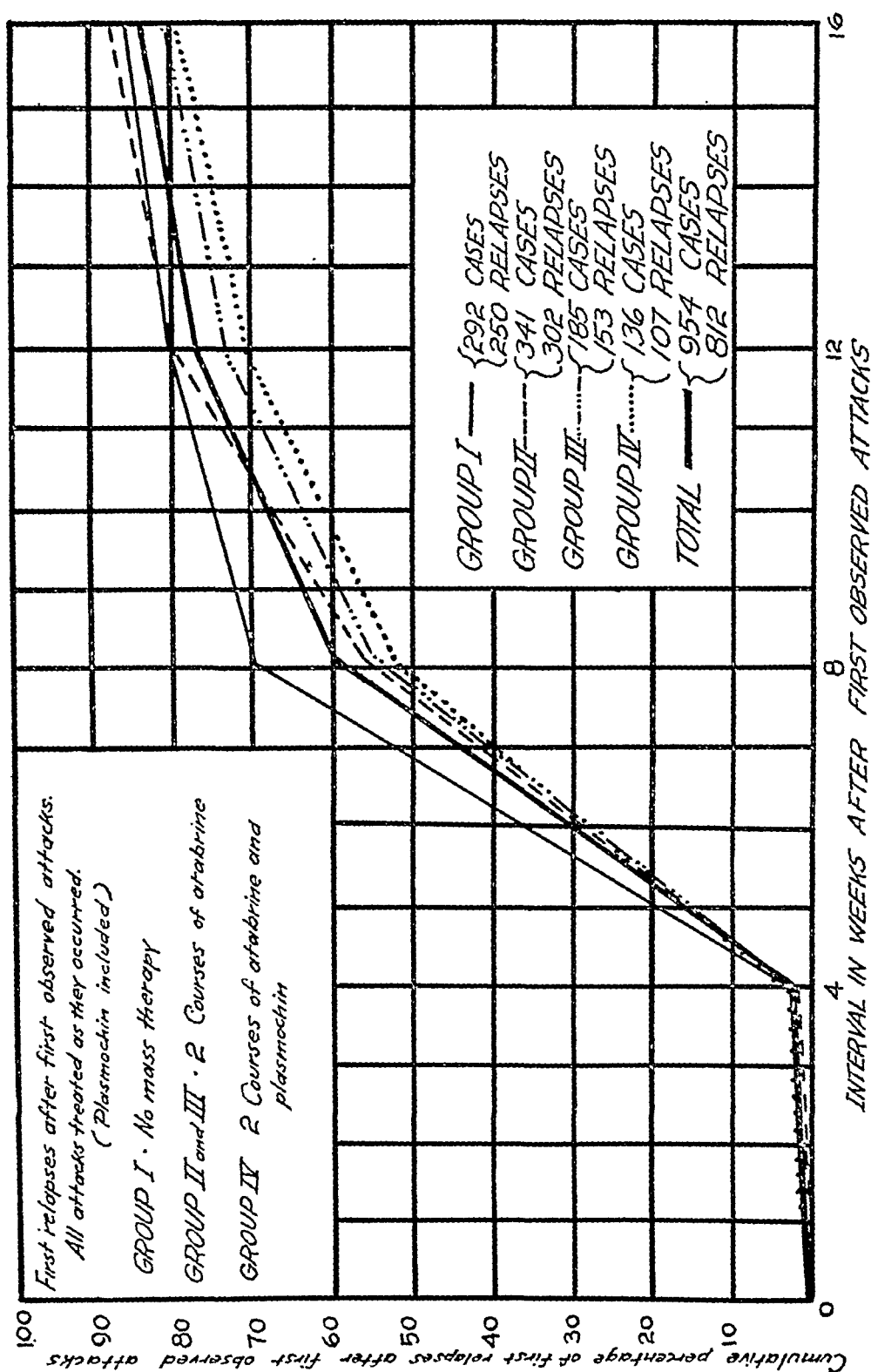


FIG. 3. INCIDENCE OF FIRST RELAPSES OCCURRING AFTER FIRST OBSERVED ATTACKS OVER A 16-WEEK PERIOD—FOR DIFFERENT GROUPS  
Expressed as percentage of first observed attacks relapsing

When first observed attacks from each group which can be followed for a period of 16 weeks after the initial attack are selected and compared, it is possible to illustrate more clearly the effects of mass therapy in the different groups.

A total of 954 first observed attacks from all groups could be followed for 16 weeks and 812, or 85.5 per cent, had relapsed by this time. None of the groups varied markedly from this, as is shown in figure 3. Group IV (which received plasmochin during mass therapy) is consistently below the level of the average for all groups and when this difference is tested for significance the final total in Group IV of 79 per cent relapses is found to vary from the combined total of all groups (85.5%) by 1.9  $\sigma$ .

Group IV	$\sigma = 3.49\%$
Observed difference (85.5% - 79%)	$= 6.5\%$
Or	$= 1.9 \sigma$

Group III (which did not receive plasmochin during the course of mass therapy), moreover, had a total of 81.8% relapses (Group III  $\sigma = 2.8\%$ ). There is no significant difference between Group III and Group IV. It appears that none of the types of mass therapy altered the number of first relapses appreciably. (The corrected rates of first relapses occurring after first observed attacks referred to above but not included in this study showed clearly that all the groups had high rates still operating on individuals available for such first relapses at the end of the time period. These rates operating for several months more would reduce available individuals in all groups about to zero and raise the cumulative total of individuals relapsing after first observed attacks in Figure III nearly to 100 per cent.)

Thus after demonstrating first that mass therapy of types employed cured no individuals of vivax malaria, it is further evident that it did not alter appreciably the later course of the disease as far as first relapses after first observed attacks are concerned.

## 2. *Effect of mass therapy on total relapses*

Study of figure 1, Groups I, II, III and IV, indicates that rates for total relapses are high in all groups at the end of the period of study, and appreciably higher in Groups I and II than in Groups III and IV.

By following initial cases in each group for a period of 16 weeks after initial attack further information can be gained on tendency to relapse. Table 3 presents these data.

There is evidenced here a tendency for fewer total relapses to occur after first observed cases in Group IV during the 16-week period of observation. Whether this represents a cutting down on number of relapses or whether it might represent merely a longer time interval between relapses with the eventual total number unaffected cannot be determined from the data.

The only difference between therapy in Group IV and that employed in Groups II and III, aside from a slight difference in time required for administration of

mass therapy, is the inclusion of plasmochin. This possible effect of plasmochin will be discussed in a separate paragraph later.

TABLE 3

*Ratio of total relapses to initial cases in different groups over a 16-week period*

GROUP	FIRST OBSERVED ATTACKS WHICH COULD BE FOLLOWED 16 WEEKS	TOTAL OF ALL RELAPSES	RATIO OF ALL RELAPSES TO ORIGINAL CASES
I	292	444	1.52
II	341	466	1.37
III	185	252	1.36
IV	136	159	1.17
Total.....	954	1321	1.38

### III. PERIODICITY OF RELAPSES

Appendix IV and figure 4 illustrate the periodicity of first relapses expressed as percentage of relapsing cases relapsing by weeks. From the figure, a very definite tendency relapse during the sixth week is evident; 30.3 per cent of relapsing cases relapsed in this week and the period of the fifth through the seventh weeks accounts for 57.1 per cent of the total number of relapsing cases.

Figure 1, Group I, illustrates clearly the periodic tendency to relapse. A high peak of first observed cases is followed in six weeks by a high peak of relapses and in five weeks by another high peak of relapses.

This periodic tendency to relapse has occurred in individuals who had at one time been on atabrine suppressive therapy and who all received quinine, atabrine and plasmochin therapy after their first observed attack. No suppressive therapy was given between attacks.

### IV. COMPARISON OF HISTORIES OF INDIVIDUALS WHO HAD MALARIA PRIOR TO INCEPTION OF STUDY WITH THOSE WHO HAD FIRST ATTACKS DURING THIS STUDY

In all of the preceding discussions first observed attacks are continually referred to, and indicate the first attack of malaria while under observation for purposes of study. Some of these individuals had had malaria previously on the malarious island; in others, there was no past history of malaria. These data were recorded for each case. The history for the group who claim no previous malaria attack is probably quite accurate. The history for the group claiming prior attacks is undoubtedly quite inaccurate, since most diagnoses on the malarious base were clinical diagnoses, and many hoped that by claiming malaria attacks they might be evacuated.

For this study 500 cases selected at random, 250 from each category above, spread equally through Groups I, II, IV, were followed for a period of 16 weeks.

Table 4 shows the close parallelism between the two groups and indicates that

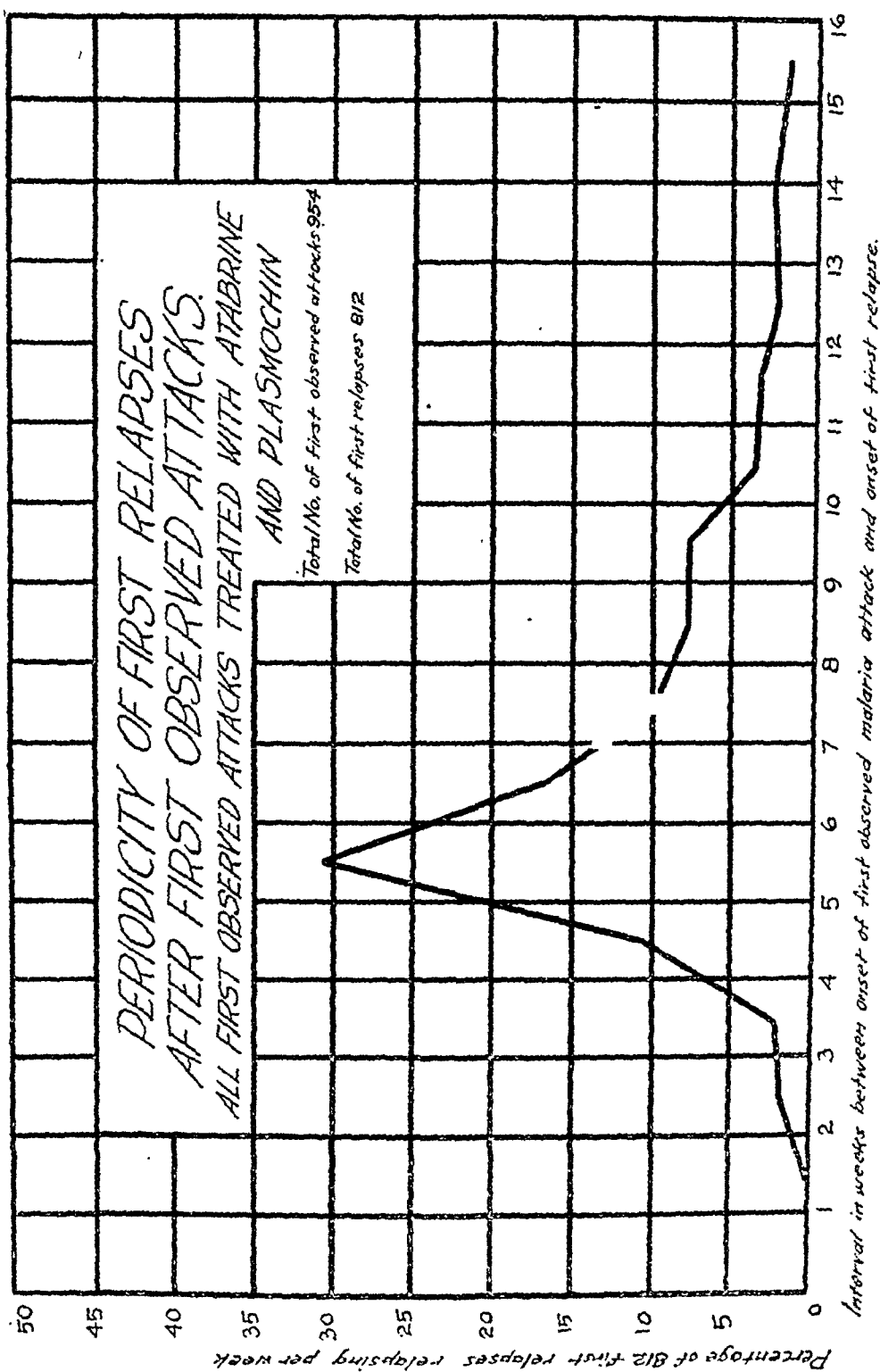


FIG. 4. PERIODICITY OF FIRST RELAPSES OCCURRING AFTER FIRST OBSERVED ATTACKS  
Expressed as percentage of relapsing cases relapsing per week—followed over 16-week period

grouping first observed cases without attempting to separate them into primary attacks or relapses should not affect calculations appreciably.

From this it would appear that a vivax malaria case occurring after atabrine suppressive therapy is discontinued behaves the same in its later course regardless of whether it is a primary case or an early relapsing case. It should be noted that the total time covered by this study, from original exposure to malaria to termination of the study, is less than one year. This generalization may not extend to groups who have been on atabrine suppressive therapy for longer periods of time or to relapsing cases later in the course of disease.

TABLE 4

*Malaria experience over a period of 16 weeks in groups with and without previous malaria attacks*

GROUP	TOTAL NO. OF CASES	1 ATTACK ONLY	2 ATTACKS ONLY	3 ATTACKS ONLY	4 ATTACKS ONLY
Malaria on malarious island.....	250	34	98	98	20
No previous malaria but developed malaria during stay on non-malarious island.....	250	27	108	103	12

#### V. EVALUATION OF PLASMOCHIN

It is difficult to arrive at a satisfactory conclusion as to what the effect of plasmochin has been in the above study. Group IV differs slightly in several respects from the other groups, with the only apparent reason for this difference being the inclusion of plasmochin in the course of mass therapy.

It can be stated with considerable certainty, after referring to figure 2 and table 1, that none of the methods used apparently cured any infections. However, figure 2 does indicate that inclusion of plasmochin prevented the early high peak rates of first observed attacks and spread the experience over a longer time period.

In considering relapses we again see that the plasmochin mass treated group has had a consistently although not markedly better experience as far as overall rates and ratio of total relapses to original cases are concerned (table 3). However, studying figure 3, it appears that over a long enough period of time plasmochin has not served to influence percentage of first relapses occurring after first observed attacks. Later relapses after the first relapse are more markedly affected to give us the differences noted in total relapses. Over a 16-week study period table 5 serves to illustrate this point. Each case in this group was followed for a total of 16 weeks after the first observed attack of malaria. Cases were selected at random.

It is to be noted that the dosage of plasmochin used was relatively small. Larger doses might have clarified the results.

This apparent effect of plasmochin in reducing the tendency to repeated relapses within the period of observation is more difficult to understand when it is

remembered that all first observed cases and relapses occurring in all groups, up until October 1, were given plasmochin routinely at the end of the treatment course, and that therefore a high percentage of the patients in first observed and earlier relapsing cases in all the groups had the benefit of this treatment.

Furthermore, even with the differences noted above as possibly due to plasmochin, the effect of this drug in preventing relapses is not so marked as to lead us to believe that its use either in suppression or routine treatment of malaria cases will appreciably improve the malaria history of an organization. A much longer period of study than the present one will be needed to settle this point

TABLE 5

*Malaria experience over 16 weeks in 500 patients from treatment groups*

GROUP	TOTAL IN GROUP, FIRST OBSERVED ATTACK	NO. WITH NO RELAPSE AFTER FIRST OBSERVED ATTACK	NO. WITH 1 RELAPSE AFTER FIRST OBSERVED ATTACK	NO. WITH 2 RELAPSES AFTER FIRST OBSERVED ATTACK	NO. WITH 3 OR MORE RELAPSES AFTER FIRST OBSERVED ATTACK
I	200	34 (17%)	66 (33%)	80 (40%)	20 (10%)
II	200	21 (10.5%)	88 (44%)	83 (41.5%)	8 (4%)
IV	100	16 (16%)	52 (52%)	28 (28%)	4 (4%)

satisfactorily. The toxicity of plasmochin is so well established that its routine use in treatment of vivax malaria does not appear justified.

#### SUMMARY AND CONCLUSIONS

It is generally recognized that strain differences exist in vivax malaria. These differences may be reflected in widely differing tendencies to relapse, and in widely differing relapse intervals. This study is concerned with the strain or strains of malaria endemic originally upon the malarious island, plus possibly other strains introduced with troops. This experience may be at variance with experiences with other strains of vivax malaria encountered elsewhere. With the strain or strains encountered, however, the following conclusions can be drawn:

1. In troops almost universally infected with vivax malaria, few or no individuals were cured of infection by suppressive atabrine therapy (0.4 gm. per week) administered for a period of six months during exposure on a malarious island.

2. Few or no cases of vivax malaria were cured by mass therapy with atabrine or with atabrine plus plasmochin after leaving the malarious island.

3. Initial attacks of malaria occurred as late as six months or more after discontinuing atabrine suppression or after mass therapy of any of the types employed.

4. Atabrine mass therapy did not alter appreciably the number of first relapses occurring after first observed attacks.

5. There was a definite tendency to relapse between the fifth and seventh week after the first observed attack, with a sharp peak in the sixth week.

6. No difference in course of disease was noted between the group which had had malaria attacks previously on the malarious island and had first observed

attacks during the course of this study and the group which had not had malaria attacks on the malarious island and had actual primary attacks during the course of study. (The total time span from date of first exposure to infection to termination of study was one year.)

7. Plasmochin administered as a part of mass therapy apparently aided in lowering the peak rate of first observed attacks, and spread the experience over a longer time period without appreciably altering the final outcome as far as first observed attacks and first relapses are concerned. There may have been a slight tendency to lower the ratio of total relapses to first observed attacks, but this point is far from being clearly established and needs observation over a longer period of time.

## APPENDIX I

*Basic statistics and uncorrected malaria rates — infantry*

WEEK	NUMBER EVACU- ATED	COR- RECTED STRENGTH	FIRST OBSERVED ATTACKS	RELAPSES	ATTACKS	RATE/1000/ANNUM		
						First observed attacks	Relapses	Total
Group I*								
5/12- 5/22 Suppression stopped 5/23	3	390	7	0	7	933	0	933
5/23- 5/29	0	390	28	0	28	3733	0	3733
5/30- 6/5	2	388	30	1	31	4020	134	4154
6/ 6- 6/12	0	388	108	3	111	14474	402	14876
6/13- 6/19	1	387	88	5	93	11824	671	12495
6/20- 6/26	1	386	26	4	30	3502	538	4040
6/27- 7/3	1	385	14	10	24	1890	1350	3240
7/4 - 7/10	3	382	12	29	41	1633	3947	5580
7/11- 7/17	0	382	4	52	56	544	7078	7622
7/18- 7/24	1	381	7	67	74	955	9144	10099
7/25- 7/31	3	378	5	33	38	687	4539	5226
8/1 - 8/7	13	365	6	30	36	854	4273	5127
8/8 - 8/14	12	353	4	30	34	589	4419	5008
8/15- 8/21	5	348	2	31	33	298	4632	4930
8/22- 8/28	0	348	1	49	50	149	7321	7470
8/29- 9/4	2	346	1	36	37	150	5410	5560
9/5 - 9/11	4	342	4	28	32	608	4257	4865
9/12- 9/18	14	328	1	31	32	158	4914	5072
9/19- 9/25	9	319	0	28	28	0	4564	4564
9/26-10/2	3	316	0	32	32	0	5265	5265
10/3 -10/9	1	315	0	23	23	0	3796	3796
10/10-10/16	3	312	3	28	31	500	5666	5166
10/17-10/23	2	310	1	32	33	167	5367	5534
10/24-10/30	0	310	0	30	30	0	5032	5032
10/31-11/6	3	307	0	29	29	0	4912	4912
11/7 -11/13	5	302	2	26	28	344	4476	4820
11/14-11/20	2	300	0	29	29	0	5026	5026
11/21-11/27	1	299	0	32	32	0	5565	5565

\* Basic data for figure 1, Group I.



# APPENDIX I—Continued

WEEK	NUMBER EVACU- ATED	COR- RECTED STRENGTH	FIRST OBSERVED ATTACKS	RELAPSES	ATTACKS	RATE/1000/ANNUM		
						First observed attacks	Relapses	Total
Group II†								
5/12- 5/22	1	689	39	0	39	2943	0	2943
5/23- 5/29	0	689	13	0	13	981	0	981
5/30- 6/5	0	689	7	0	7	528	0	528
6/6 - 6/12	0	689	14	2	16	1056	150	1206
6/13- 6/19	1	688	6	0	6	453	0	453
Stopped mass treatment 6/17								
6/20- 6/26	5	683	5	4	9	380	304	684
6/27- 7/3	0	683	14	8	22	1065	609	1074
7/4 - 7/10	6	677	40	10	50	3072	768	3840
7/11- 7/17	0	677	85	13	98	6528	998	7526
7/18- 7/24	4	673	130	14	144	10044	1081	11125
7/25- 7/31	3	670	72	7	77	5588	543	6131
8/1 - 8/7	1	669	46	13	59	3575	1010	4585
8/8 - 8/14	11	658	27	15	42	2133	1185	3318
8/15- 8/21	10	648	20	38	58	1604	3049	4653
8/22- 8/28	6	642	18	41	59	1457	3320	4777
8/29- 9/4	6	636	14	60	74	1144	4905	6049
9/5 - 9/11	0	636	5	44	49	408	3597	4005
9/12- 9/18	18	618	10	44	54	841	3702	4543
9/19- 9/25	4	614	5	45	50	423	3811	4234
9/26-10/2	5	609	5	39	44	426	3330	3756
10/3 -10/9	4	605	4	47	51	343	4039	4382
10/10-10/16	3	602	4	42	46	345	3627	3972
10/17-10/23	6	596	5	40	45	436	3489	3925
10/24-10/30	2	594	2	31	33	175	2713	2888
10/31-11/6	4	590	0	52	52	0	4583	4583
11/7 -11/13	6	584	3	30	33	267	2671	2938
11/14-11/20	1	583	6	54	60	535	4816	5351
11/21-11/27	3	580	3	62	65	268	5538	5826

† Basic data for figure 1, Group II.

## Group III†

5/12- 5/22	0	456	16	0	16	1785	0	1785
5/23- 5/29	0	456	43	0	43	4903	0	4903
5/30- 6/5	0	456	24	0	24	2736	0	2736
6/6 - 6/12	0	456	4	0	4	456	0	456
6/13- 6/19	1	455	2	1	3	228	114	342
6/20- 6/26	5	450	2	5	7	235	577	812
Stopped mass treatment 6/27								
6/27- 7/3	0	450	5	6	11	577	693	1270
7/4 - 7/10	5	445	10	6	16	1168	701	1869
7/11- 7/17	0	445	18	13	31	2103	1519	3622
7/18- 7/24	6	439	39	6	45	4619	710	5329
7/25- 7/31	2	437	52	11	64	6306	1308	7614
8/1 - 8/7	2	435	72	13	85	8602	1554	10156

† Basic data for figure 1, Group III.

# APPENDIX I—Continued

WEEK	NUMBER EVACU- ATED	COR- RECTED STRENGTH	FIRST OBSERVED ATTACKS	RELAPOSES	ATTACKS	RATE/1000/ANNUM		
						First observed attacks	Relapses	Total

## Group III—Continued

8/8 - 8/14	10	425	35	9	44	4282	1101	5383
8/15- 8/21	8	417	12	20	32	1496	2494	3990
8/22- 8/28	1	416	16	26	42	2000	3250	5250
8/29- 9/4	5	411	6	21	27	759	2656	3415
9/5 - 9/11	0	411	7	26	33	885	3289	4174
9/12- 9/18	13	398	9	32	41	1175	4180	5355
9/19- 9/25	4	394	3	33	36	395	4355	4750
9/26-10/2	2	392	2	18	20	265	2387	2652
10/3 -10/9	8	384	5	17	22	677	2303	2979
10/10-10/16	3	381	2	34	36	272	4640	4912
10/17-10/23	1	380	2	32	34	273	4378	4651
10/24-10/30	1	379	2	26	28	274	3567	3841
10/31-11/6	4	375	1	32	33	138	4437	4575
11/7 -11/13	0	375	3	32	35	416	4437	4853
11/14-11/20	0	375	3	31	34	416	4298	4714
11/21-11/27	1	374	4	24	28	556	3336	3892

## Group IV§

5/12- 5/22	0	457	19	0	19	2161	0	2161
5/23- 5/29	1	456	12	0	12	1368	0	1368
5/30- 6/5	0	456	3	0	3	342	0	342
6/6 - 6/12	3	453	0	0	0	0	0	0
6/13- 6/19	3	450	1	0	1	115	0	115
6/20- 6/26	1	449	1	1	2	115	115	230
6/27- 7/3	0	449	5	0	5	579	0	579
Stopped mass treatment 7/2								
7/4 - 7/10	11	438	5	2	7	593	237	830
7/11- 7/17	0	438	9	3	12	1068	356	1424
7/18- 7/24	2	436	35	6	41	4174	715	4889
7/25- 7/31	4	432	52	2	54	6259	240	6499
8/1 - 8/7	1	431	41	5	46	4946	603	5549
8/8 - 8/14	13	418	34	6	40	4229	746	4975
8/15- 8/21	6	412	30	4	34	3786	504	4290
8/22- 8/28	5	407	29	12	41	3705	1533	5238
8/29- 9/4	6	401	17	24	41	2204	3112	5316
9/5 - 9/11	0	401	14	24	38	1815	3112	4927
9/12- 9/18	7	394	12	16	28	1583	2111	3694
9/19- 9/25	8	386	9	20	29	1212	2694	3906
9/26-10/2	0	386	8	14	22	1077	1886	2963
10/3 -10/9	3	383	7	23	30	950	3122	4072
10/10-10/16	3	380	6	23	29	821	3147	3968
10/18-10/23	3	377	11	29	40	1517	4000	5517
10/24-10/30	3	374	5	32	37	695	4449	5144
10/31-11/6	1	373	2	25	27	278	3485	3763
11/7 -11/13	0	373	1	21	22	139	2927	3066
11/14-11/20	0	373	2	20	22	278	2788	3066
11/21-11/27	0	373	6	27	23	836	3764	4600

§ Basic data for figure 1, Group IV.

## APPENDIX II\*

*Malaria in — Infantry: corrected attack rates for first observed attacks, based on individuals available for such attacks*

WEEK	RATE PER 1000 PER ANNUM		
	Group I	Group II	Group IV
5/23- 5/29	3802	1038	1424
5/30- 6/5	4394	570	367
6/6 - 6/12	17387	1153	0
6/13- 6/19	21284	505	124
6/20- 6/26	10730	427	125
6/27- 7/3	7280	1215	629
7/4 - 7/10	7341	3555	637
7/11- 7/17	4650	8200	1189
7/18- 7/24		17094	4691
7/25- 7/31		11663	7704
8/1 - 8/7		9684	7158
8/8 - 8/14	2771	7020	6879
8/15- 8/21		6010	7189
8/22- 8/28		6198	8195
8/29- 9/4		5515	5616
9/5 - 9/11	2408	2203	5556
9/12- 9/18		4601	5333
9/19- 9/25		2626	4500
9/26-10/2		2795	4654
10/3 -10/9	2476	2437	5524
10/10-10/16			
10/17-10/23			
10/24-10/30			
10/31-11/6	1651	2313	3213
11/7 -11/13			
11/14-11/20			
11/21-11/27			

\* Basis data for figure 2.

## APPENDIX III\*

*Malaria in — infantry: per cent of first relapses in sixteen-week period after first observed attack*

GROUP	FIRST OBSERVED ATTACKS	PER CENT OF FIRST OBSERVED ATTACKS RELAPSING FOR FIRST TIME				
		1-4 weeks	5-8 weeks	9-12 weeks	13-16 weeks	Total
I	292	3.1	66.1	12.3	4.8	86.3
II	341	1.5	54.5	24.3	8.2	88.2
III	185	2.2	52.9	18.9	8.8	81.8
IV	136	3.3	49.2	19.1	7.4	79.0
Total.....	954	2.3	56.8	18.8	7.2	85.5

## APPENDIX IV\*

*Malaria in — infantry: periodicity of 812 first relapses after 954 first observed attacks, expressed as per cent of cases relapsing per week*

The 812 relapsing cases relapsing per week

WEEK	GROUP I	GROUP II	GROUP III	GROUP IV	TOTAL	PER CENT
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	2	3	2	1	8	1.0
4	7	2	2	3	14	1.7
5	42	18	16	8	84	10.3
6	91	84	39	32	246	30.3
7	40	56	20	18	134	16.5
8	18	28	23	9	78	9.6
9	9	34	10	10	63	7.8
10	16	29	12	7	64	7.9
11	6	11	8	4	29	3.6
12	5	9	5	5	24	2.9
13	4	7	3	3	17	2.1
14	4	8	4	3	19	2.3
15	3	6	6	2	17	2.1
16	3	7	3	2	15	1.8

\* Basic data for figure 4.

# THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

## I. THE SYMPTOMS, SIGNS AND PHYSICAL FINDINGS CHARACTERISTIC OF SCHISTOSOMIASIS JAPONICA AT DIFFERENT STAGES IN THE DEVELOPMENT OF THE DISEASE

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### INTRODUCTION

The first clinical report of schistosomiasis japonica, referred to under the name "Katayama disease", was published in 1847 (1), more than half a century before the etiologic agent was discovered (2). In 1883, Baelz (3) described enlarged liver and spleen, bloody diarrhea, anemia, fever, cachexia, ascites and edema as characteristic of the disease. For the next twenty five or thirty years Japanese physicians and pathologists were impressed with the fever which was at times confused with that of malaria (4); the hypertrophy of liver and spleen, especially as observed in young people (5); later the cirrhosis of the liver (6) with ascites (7); the anemia (8); the frequent association of the disease with appendicitis (9), carcinoma of the liver (10) or bowel (11, 12), or with Jacksonian epilepsy (13, 14).

The earliest reports of schistosomiasis in China appeared a few months after its etiology was clarified (15, 16). Here within a few years American and British physicians came to recognize in their own nationals, who were occasionally exposed to infection by bathing or swimming in the Yangtze river or its tributaries (17), certain relatively early pathognomonic symptoms and signs, particularly urticaria (18, 19), pseudo-angioneurotic edema (20) and eosinophilia (17, 19) accompanied by intermittent evening fever (19).

The discovery of the disease in the Philippines in 1906 (21) soon added valuable epidemiological and pathological information, but essentially no new clinical data. Similarly, knowledge that the disease was mildly endemic on Formosa (22) produced no significant clinical contribution.

### STAGES OF INFECTION OBSERVED

The Japanese medical literature from 1883 for a period of approximately four decades contains a wealth of information on the etiology, epidemiology, pathology and attempted control of schistosomiasis. There are likewise a large number of

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clinical observations on the chronic stage but essentially no information on the incubation period and the onset of the disease.

In China the great majority of patients suffering from schistosomiasis have been natives who came to dispensaries and hospitals in a moderately advanced or late chronic stage of the infection. When foreigners occasionally exposed themselves accidentally by wading in infested swamps, or swimming in infested bodies of water in the Yangtze valley, it was possible to observe the disease during its developmental period. These relatively few observations have been recorded by Lambert (18), Laning (23), and Faust and Meleney (24) on British and American naval personnel, by Logan (20) on an American youth and by Lee (25) on a group of Roumanian gypsies. In the summer of 1923 a moderate sized epidemic of schistosomiasis was discovered in a single group in China, who were exposed simultaneously and developed acute manifestations at approximately the same time (26). They consisted of about forty Chinese school boys and their American athletic director, who swam in infested water at Anking, Anhwei Province during the middle of June. A month later Dr. Harry Taylor, the missionary physician at the station, was called back from vacation to diagnose and administer treatment to the entire number, most of whom were already seriously ill. Some years later (1931) a delayed summer flood in the lower Yangtze valley reached the "backdoor" of Shanghai and produced infection in several hundred Chinese and in forty to fifty Europeans. There are no reports on the native cases but Kastein (27) has presented a graphic picture of the acute stage in fifteen of the infected Europeans. The Chinese patients seen by Logan (16), Houghton (28), Tootell (29), Faust and Meleney (24), Meleney, Faust and Waskell (30), Chu (31), as well as other investigators in China, had been exposed months to years earlier or had been subject to a series of exposures superimposed on one another year after year.

In the Philippines there were many cases of schistosomiasis diagnosed each year among the inmates of Bilibid Prison, but until recent years there have been no reliable survey records of the infection among the native population in endemic foci in these Islands (32, 33).

The pathogenesis of schistosomiasis japonica during the earlier stages has been elucidated exclusively from studies on experimentally infected laboratory animals, which were exposed at known times to varying amounts of inoculum and were sacrificed from day to day, in order to trace the migration of the developing worms and the lesions which they produced (24). This has served a very useful purpose in building up a knowledge of the disease process to a point where human autopsy material was available to carry the picture to its terminal stage. The experimental material has likewise indirectly provided a pathological basis for the anticipated symptoms, signs and physical findings in man from the time of exposure to the characteristic, well matured picture of the disease.

#### CASE MATERIAL UTILIZED

The occurrence of several hundred cases of schistosomiasis japonica among military personnel within a limited period of months following the American

reoccupation of Leyte, Philippine Islands, beginning in October, 1944, has provided a group of patients with this disease who were available for study during the late incubation, acute and early chronic stages. For comparison there have been many natives of all ages on Leyte, Mindoro, Mindanao and Samar, who have been exposed to single or multiple infections and usually exhibit a relatively advanced stage of the disease.

The case material which has been available for the present study has consisted of the following:

1. Certain military cases in the wards of an American Army General Hospital on Leyte, P. I.
2. A group from a Royal Australian Air Force Construction Squadron, who had been exposed for a brief period on Leyte.
3. Native cases in *Union barrio*, Leyte.
4. Native patients in the San José Civilian Hospital, Leyte.
5. Native patients seen in the Valencia Civilian Hospital and in a nearby Evacuation Hospital, Mindanao.
6. Native civilian and guerrilla patients in an American Field Hospital, Leyte.

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## DEVELOPMENT OF SYMPTOMS AND PHYSICAL FINDINGS

*Initial factors involved.* Before an attempt is made to present a picture of the progressive clinical findings in schistosomiasis japonica it is necessary to indicate that these findings vary quantitatively and qualitatively in different patients, depending on several factors. The more significant of these will be briefly outlined.

*Amount of inoculum in infested water at time of exposure.* The inoculum consists of the viable fork-tailed larvae, the cercariae, capable of penetrating human skin and initiating an infection. The quantity of the inoculum differs in endemic areas seasonally and in different locations within an endemic focus, due to the number of cercariae released by the snail hosts, the distance of the site of exposure from the snail source of supply for the cercariae, whether the water is stagnant or flowing, and the age, hence the invasive capacity, of the cercariae. These are all biological and epidemiological factors which are important in determining the dosage of inoculum to which the individual is exposed, hence they are clinically important.

*Area of skin (and mucous membrane) exposed.* Wading only ankle-deep in infested water, especially if pants are carefully tucked inside well-laced combat boots, provides relatively scant opportunity for exposure. Wading up to the armpits and bathing or swimming in infested water constitute heavy exposure hazards. Contact of infested water with the foreskin, or the mucous membranes of the mouth and anus, is especially dangerous, since the cercariae are able to enter these tissues much more easily than the more cornified epidermis.

*Single or repeated exposure.* Single exposure, particularly if it extends over a period of hours, may provide sufficient inoculum for a fulminating infection. On the other hand, infection may be built up to an equally high level by daily exposures of much shorter duration.

*Reaction of patient to the parasite.* The metabolites released by the invading parasite during its migration through the tissues, its period of growth and maturing, and the tissue reaction to the eggs which are laid by the mature worms daily for weeks, months and even years, invariably produce physical changes and symptoms in the patient. If few worms enter the body, the reaction will usually be minimal; if many worms penetrate the skin, the reaction will be quantitatively greater. Similarly, if few worms reach maturity, correspondingly few eggs will be laid and pathological changes due to this process will be relatively slight, possibly of subclinical importance. If many worms reach maturity, the acute reaction to the worms and their eggs may be fatal to the patient. Nevertheless, all individuals probably do not react in the same degree to equal amounts of inoculum. Observation suggests that small native children are particularly sensitive to schistosomiasis japonica and that some military personnel have reacted in a comparable manner.

## EARLY MANIFESTATIONS AND FINDINGS

The greatest difficulty in interpreting the early signs and symptoms of schistosomiasis japonica is the uncertainty as to whether they are due to this or to



several other diseases which may have more or less similar manifestations. Within a few minutes after skin exposure to schistosomiasis japonica a person may experience sharp needling pain at the sites of penetration of the fork-tailed cercariae, followed from one to several days later by an irritating, frequently unproductive bronchial cough of a few days' duration, or at times a viscous mucous discharge from the lungs. There may then be no additional distinct symptoms for two weeks or longer, when overnight a moderate to severe urticaria may develop.

For the first time the patient suddenly begins to realize that he is sick; he is losing his appetite; he tires easily; he is developing headache and mental dullness and is beginning to lose weight. He becomes gradually aware of a fullness in the epigastric region and tenderness on pressure in the upper part of the abdomen directly to the right of the xiphoid process. Towards evening he first feels chilly, then his skin feels hot and he has a tendency to sweat at night, although next morning he feels nearly normal, except for moderate weakness. These symptoms increase slowly until in about four to five weeks after exposure there is pain and fullness in the right hypochondrium, general abdominal distress, frequently mucous diarrhea and more or less profound anorexia. Suddenly there may be a painful bowel movement and flecks of bloody mucus will be discharged in the stools.

The symptoms which have been described above, essentially in their proper sequence, seldom all occur in any one patient. Some individuals give a clear history of netting dermatitis within a few minutes to a few hours following exposure (Lee, 25); on the other hand, many do not, while others will recall some kind of skin irritation following wading or bathing in fresh water, but their information is so indefinite that the dermatitis might have been due to any one of several irritating agents. The bronchial cough is suggestive only when it follows within a few days a definite exposure in infested water, with or without the netting dermatitis. These conditions have usually been forgotten before the urticaria develops. Moreover, urticaria may not occur or it may be so slight as to pass unnoticed. Again, it may appear only after the disease has passed from the incubation period well into the stage of acute illness. Thus, none of the above manifestations are likely to suggest that the individual is developing blood-fluke infection unless the attending physician is experienced with the disease.

The physical weakness and mental lethargy, loss of appetite and loss of weight, pain and stiffness in the leg muscles, small of the back and neck, the headache, the intermittent chilly sensation followed by fever each night, the abdominal fullness and tenderness in the right hypochondrium,—all of these are relatively constant features of the disease as it approaches the end of the incubation period.

Physical examination at this time will usually reveal no abnormalities of the head, neck, heart, lungs or reflexes resulting from the infection, except for nuchal rigidity which may appear this early. On deep inspiration there will be acute tenderness under the ribs immediately to the right of the midventral line, and as the disease progresses an exquisitely tender liver may be palpated, or at least demonstrated by percussion. This syndrome may suggest infectious hepatitis,

leptospirosis, relapsing fever, or may be mistaken for amebic hepatitis or amebic liver abscess.

The intestinal symptoms, including indigestion, loss of appetite, tenderness throughout the length of the small bowel, possibly a syndrome of acute or sub-acute appendicitis, and mucous diarrhea are all moderately constant prodromes of intestinal schistosomiasis, but they may be, and have been, confused with typhoid or paratyphoid fever, undulant fever, influenza or hookworm disease.

A blood count made towards the end of the incubation period will usually reveal a polymorphonuclear leukocytosis with notable eosinophilia, but no significant change in the red cells.

Unless the entire picture of the incubation period is obtained in sequence, there is not a sufficiently clear train of symptoms to make a satisfactory presumptive diagnosis of intestinal schistosomiasis, although a history of having bathed in fresh water in an endemic area about four or five weeks previously will add considerable strength to a diagnosis based on the more constant characteristic features of this stage of the infection. Moreover, it must be emphasized that many skilled medical officers, who had been indoctrinated with information concerning the endemicity of the disease on Leyte but without previous actual experience, failed to recognize the signs and symptoms of the disease before the acute stage had been reached. Unfortunately there is no laboratory aid which has yet been developed that will provide specific confirmation of a clinical diagnosis of this infection during the incubation period.

Some lightly exposed individuals may react clinically to a minimum infection, and may manifest urticaria, fever, malaise, muscle pains, epigastric pain and hepatic tenderness. Probably the majority of light infections, however, will pass through an essentially symptomless incubation period and the disease will remain undiagnosed for months or years, unless routine stool examination for parasites is made by a competent laboratory worker. On the other hand, physicians inexperienced with this disease, have, on the basis of certain suggestive symptoms and physical findings, arrived at a clinical diagnosis of schistosomiasis japonica and have even proceeded to specific treatment without demonstration of the egg of *S. japonicum* or have undertaken treatment on the basis of mistaken laboratory diagnosis (i.e., plant-cell artifacts diagnosed as eggs).

#### THE ACUTE STAGE

The onset of the acute stage occurs at the time the mature, fertilized female worms are beginning their egg-laying and the eggs are filtering through the intestinal wall, usually to be evacuated in the stool within a period of a few days. This stage may be expected to last for approximately three to four months, by which time it will gradually be transformed into the early chronic condition. Four different varieties of this stage may be distinguished, viz. 1) fulminating, 2) severe with sudden onset, 3) insidious and 4) asymptomatic. In addition, 5) complications must be considered.

(1) *Fulminating infections.* While this type has undoubtedly occurred from time to time, the patient has heretofore probably died before specific diagnosis

was made. At least one instance of infection in a military patient belongs to the category of fulminating schistosomiasis. Before the end of the incubation period the patient was seriously ill, with evidences of acute systemic intoxication, a greatly enlarged tender liver and abdominal distress. Several stool examinations were negative for eggs of *S. japonicum* but the condition was so strongly suggestive of the disease that tartar emetic treatment was begun. The patient tolerated the treatment very poorly, his condition became critical and death occurred shortly after the second administration of the drug. Necropsy revealed the accuracy of the clinical diagnosis, with myriads of eggs in pseudo-abscesses and pseudo-tubercles studding the surface and substance of the liver and other organs and tissues. It seems probable that exposure to infection had been extremely heavy, that reaction to this massive infection had been very severe, and that death had resulted primarily from an overwhelming intoxication produced by the metabolites of the worms and their eggs.

(2) *Severe, with sudden onset.* This is the variety of the disease which Faust and Meleney (24) observed in an American Naval officer, which Lee (25) described for two of his family group of patients and which Kastein (27) reported for several of his fifteen cases. In Kastein's series the prodromal symptoms consisted of fatigue, mental apathy, severe headache, early evening fever ranging from 38° to 41°C., loss of appetite, nausea, an unexplained feeling of heaviness in the abdomen, tachycardia and a desire to cough but without bronchitis. Urticaria appeared in every instance a few days after the onset. Physical examination revealed clear lungs and heart, although there was an accentuation of the T wave. The reflexes were normal. Neither liver nor spleen was palpable, possibly because none of the individuals had been subject to heavy exposure and the infection had not yet developed its full picture. Contrary to the usual condition at the onset of acute symptoms in schistosomiasis japonica, the stools were constipated and were never bloody. In each instance, however, eggs of *Schistosoma japonicum* were found in numbers in the feces. There was a leukocytosis ranging from 18,000 to 28,000 and an eosinophilia of 42 to 72 per cent. The presence of hookworms and whipworms, as indicated by eggs in the stools of some of the patients, may have contributed somewhat to the blood picture but was not sufficient to explain the unusually high eosinophilic leukocytosis. Cultures of the blood, sputum and urine were all negative for pathogenic organisms. The incubation period was calculated to be four to six weeks, agreeing essentially with the estimate of Faust and Meleney (24), Lee (25) and Taylor (26).

In the Leyte military series the first symptoms in one group of eight of a Company of Engineers, whose exposure consisted of swimming once in infested water, appeared four to five weeks later. In another Company of the same Corps, whose exposure was heavy and prolonged during bridge construction, symptoms appeared in some individuals within a somewhat shorter time, although the median date of first symptoms was the same, i.e., 4 to 5 weeks following exposure. The earliest hospital sheet entries in these positive military cases before laboratory diagnosis of schistosomiasis japonica had been made included: fever of unexplained origin, urticaria, angioneurotic edema, gastro-

intestinal distress, acute diarrhea, dysentery, dengue, suspected infectious hepatitis and upper respiratory infection.

Within 81 days following the first possible exposure 61 per cent of the schistosomiasis cases in an Engineering Battalion had been hospitalized because of severe illness. Seventy seven per cent of this positive series had an eosinophilia of 30 per cent or over and only 6 per cent of the total had less than 10 per cent eosinophilia. One patient had 90 per cent eosinophilia. While some of these cases had hookworm infection, strongyloidiasis, ascariasis and amebiasis, the disease from which the individuals in this series were suffering was schistosomiasis.

Clinical papers have been prepared and have already been published (34) or are to be published on the American military cases of schistosomiasis japonica. These contain detailed accounts of the symptoms and physical findings of the patients. In this more general consideration of the subject it is sufficient to state that all of the previously described elements in the picture of the acute stage of the disease were encountered, essentially in the same sequence and varying according to the degree of exposure and reaction of the patient to the parasite and its products. Even after four or five months of hospitalization several of the patients who had been under fuadin treatment totalling 40 to 80 cc. were still under weight, were apathetic and had a distressed hepatic facies. They complained of failure to regain their appetite, of continued abdominal fullness and pain, of irregular bowel movements, of muscular aches and pains and of arthralgia. Some of the men had a palpable tender liver and enlarged spleen, although in no instance was there evidence of hepatic cirrhosis or of the tremendous splenomegaly observed at a later stage in untreated infected natives.

Two relatively common findings in the American military series have not previously been described for the acute stage of schistosomiasis japonica, namely, nuchal rigidity and the development of "yellowish nodules" on the mucosal surface of the lower sigmoid colon and proximal level of the rectum.

The soreness and stiffness of the posterior neck muscles were present in so many of the schistosomiasis patients studied in a General Hospital, that the Commanding Officer, Col. James Bordley, 3rd, regards this as quite typical of the series, and pathognomonic of this stage of the disease. The rigidity was particularly noticeable in an attempt to move the head from side to side.

"Yellowish nodules" were seen with relative frequency in patients who were proctoscoped during this period. They consist of slightly elevated papillae, about one to 3 mm. in diameter and are most commonly observed from a few centimeters above the junction of the sigmoid colon and rectum to a few centimeters below this level. Biopsy of the lesions and their examination under the microscope, either in section or smear, has demonstrated that they contain nests of *S. japonicum* eggs in pseudo-abscesses or developing pseudo-tubercles. Even when the nodules can not be demonstrated, at times there are dilated blood capillaries, which on biopsy or scraping are found to contain one or more eggs. It is understandable that eggs should be present in the intestinal mucosa at this stage of the disease, for it is from this location that they are discharged into the intestinal lu-

men and are soon thereafter evacuated in the stool. Previously, however, on the basis of schistosomiasis japonica infections in experimental animals, it has been concluded that in the average infection during the acute stage the mature worms and the lesions for which they and their eggs are responsible are located primarily in the portion of bowel drained by the upper branches of the superior mesenteric vein. Later, as the disease process progresses, the drainage of the colon and rectum becomes similarly involved, with dilatation of the capillaries, the development of papillomata and even prolapsus recti (24). The relatively common observance of "yellowish nodules" or distended capillaries in the lower sigmoid colon and upper rectal levels in the American military cases indicates that the conclusion based on findings in experimental animals was somewhat misleading.

(3) *Insidious infections.* These are cases in which there is no sudden onset but a gradual development of the infective process over a period of one to three months beyond the average incubation period. There is a gradually increasing malaise; a slight increase in evening temperature which is not usually apparent to the patient as a distinct "fever" but may be mistaken for mild chronic falciparum malaria, undulant fever or leptospirosis; the gradual development of abdominal fullness and of digestive disturbance, with alternating constipation and loose stools, rarely with macroscopic blood and mucus; an almost imperceptible decrease in appetite and perhaps some loss in weight and in strength. There may be an eosinophilia of 10 per cent or higher but more frequently it is below 5 per cent and the total leukocyte count is not typically increased. Only after the disease has progressed for several months does the patient realize that he is ill.

This variety of the disease has its parallel in insidious amebic colitis. It is relatively common in schistosomiasis mansoni but has rarely been encountered in natives infected with *Schistosoma japonicum*, because of the usual complication of superinfection. Several cases have been observed in the American military series and some in the Australian Air Force Command. On the basis of histories obtained from the latter group there is suggestive evidence that slow development of the disease to clinical level is the result of light exposure associated with relatively slight sensitivity of the individual to the invading parasites.

In the first place, the clinical importance of this variety of schistosomiasis japonica consists in the difficulty of arriving at a clinical diagnosis because of the absence or low grade character of the usual pathognomonic landmarks. In a high percentage of cases the diagnosis is made only after eggs of *S. japonicum* have been reported in the stools. In the second place, by the time the etiology of the disease has been discovered the underlying pathological processes are with equal insidiousness transforming from an acute to a chronic condition and permanent tissue damage will soon be underway. Thus, specific diagnosis and treatment are important.

(4) *Asymptomatic infections.* In these cases no symptoms or signs of clinical grade appear within a period of several months following exposure. An appreciable number of this variety have turned up on routine fecal examination in American troops on Leyte or after the men had been detailed elsewhere, and during a special survey conducted by the Commission on Schistosomiasis on

treated and untreated Royal Australian Air Force personnel several months after they had left endemic territory. Questioning these individuals supplemented by physical examination provided no evidence that they were constitutionally affected by the parasite. For the time being they belong to the category of "carriers". The possible development of these cases to clinical grade at some time in the future is a matter of considerable importance.

(5) *Complications.* The complications which have been observed in schistosomiasis japonica consist in (a) extension of the lesions from the abdominal viscera to other organs and tissues of the body, and (b) secondary infections or disease processes.

(a) *Extension of schistosomiasis lesions. The lungs.* From time to time experimental animals and human cases reveal at autopsy the presence of pseudo-tubercles around eggs of *Schistosoma japonicum*, *S. mansoni* and *S. haematobium*, which have been carried from the mesenteric-portal vessels or vesical venules *via* collateral venous circulation through the right heart into the pulmonary arteries and have filtered out into the pulmonary parenchyma. During life these lesions in sufficient amount may produce râles. X-ray pictures indicate several to many small scattered areas of infiltration similar to the findings in miliary tuberculosis. At least one American military case of this type has been reported (34).

*End-arterioles.* By a mechanism as yet unexplained *Schistosoma* eggs at times get out of the closed venous-portal or the vesical-venous system (and their extensions *via* the inferior vena cava and right heart to the pulmonary arterioles) and enter the systemic arterial circulation. They are carried to sites of lodgment in end-arterioles, where they may occlude the vessels and give rise to embolic manifestations, or may filter out into perivascular tissues and produce pseudo-abscesses and eventually pseudo-tubercles. Three different anatomical locations have thus far been described.

*The toes.* In one case reported by Thomas and Gage (34) the skin of the small and great toes of both feet showed evidence of embolic occlusion of the end-arterioles. There was a black gangrenous area about 1 cm. in diameter on the outer aspect of the end of each small toe, which was exquisitely tender, while the great toes had larger dark red areas. The patient was passing *S. japonicum* eggs in his stools and had neurological complications, attributed to the disease process.

*Cutaneous lodgment of eggs.* One American military patient with this complication has been studied by Fishbon (35). Multiple lesions first appeared in the abdominal skin and were proven by scrapings to be due to *S. japonicum* eggs. Shortly thereafter similar, slightly elevated papular lesions developed in the intercostal spaces; their etiology was confirmed by biopsy.

*Neurological manifestations.* Japanese physicians (13, 14) have frequently reported Jacksonian epilepsy in association with helminthic infections. Some of these have been demonstrated to be caused by lodgment of *S. japonicum* eggs in the brain; more often they have been due to the adult worms and eggs of the lung fluke, *Paragonimus westermani*, at other times to cysticerci of *Taenia solium*. Africa and his associates (36) have found that eggs of the minute intestinal fluke, *Metagonimus yokogawai*, and its relatives may reach the brain and spinal cord and set up grave neurological disturbances.

Chu (31) has reported one case of Jacksonian epilepsy and one case of hemiplegia in his series of 39 Chinese cases of schistosomiasis japonica. In the latter instance the patient had bathed daily in a small stream from August 13 to September 19. Beginning the middle of September he developed late afternoon chills and fever. On October 12, when he was examined, he had a statue-like facies and short, stuttering speech. His spleen and liver were palpable and *S. japonicum* eggs were present in his feces. His white count was 14,500 and his eosinophils, 53 per cent. Three days later he had developed almost complete paralysis of his right extremities. Recovery from the neurological symptoms was rather prompt following the administration of 64.5 cc. of fuadin.

In addition to the two American military patients with neurological manifestations complicating intestinal schistosomiasis, reported by Thomas and Gage (34), several others have been studied. Three of these were demonstrated to one of the members of the Commission on Schistosomiasis (E. C. F.) by Col. James Bordley. One who had characteristic symptoms of intestinal schistosomiasis (viz. febrile onset, headache, nuchal rigidity, giant urticaria, hepatomegaly and splenomegaly, and loss of weight) developed neurological manifestations eleven days after the acute onset. These consisted of weakness of the muscles of the left arm, with Hoffman reflex, and transient weakness of the muscles of the left leg and left side of the face. Although the symptoms began to improve 5 days after fuadin treatment was instituted, when the patient was demonstrated three weeks later, he still had palpable liver and spleen, moderate epigastric distress, nuchal rigidity, exaggerated biceps reflex of the left arm, weakness and adiadokokinesis of the left arm, and difficulty in standing because of lack of muscular balance of the left leg.

A second schistosomiasis patient, when examined five weeks after exposure, had cough, late afternoon fever, was drowsy, had moist râles at the base of the left lung, a palpable spleen and a total white blood count of 22,050 with 38 per cent eosinophils. Nine days later he had flaccid paralysis of the left arm with exaggerated reflexes and Hoffmann reflex. In spite of fuadin therapy, when he was demonstrated five weeks after the onset of symptoms, he was markedly emaciated, was still febrile every evening and had right ankle clonus and exaggerated reflexes of the right leg. These manifestations demonstrate the existence of an early lesion in the right motor cortex and a subsequent one in the left cortex.

The third schistosomiasis patient developed neurological symptoms six and one-half months after exposure and five and one-half months after the onset of intestinal symptoms. He had been treated with two courses of fuadin, the first consisting of 40 cc., the second of 60 cc. When he was demonstrated four weeks subsequent to the first appearance of neurological symptoms he had marked weakness of the muscles of the left side of the body, numbness of the left upper lip, and left third, fourth and fifth fingers. On arising from his bed in an attempt to show the partial paralysis of his left leg, he had a Jacksonian seizure of short duration, with extreme weakness and dyspnea. Thus, lesions were demonstrated for both the right motor and sensory neurons.

(b) *Supervening diseases.* These complications may consist of chronic processes from which the patient has suffered for years, as tuberculosis, malaria,

amebic colitis, hookworm disease, strongyloidiasis, ascariasis, or deficiency disease. On the other hand, pneumonia, typhoid and paratyphoid fevers, infectious hepatitis, bacillary dysentery, amebic hepatitis or other acute infectious diseases may supervene. The members of the Commission on Schistosomiasis have seen all of these diseases complicating schistosomiasis in the Philippines, either in natives or American military patients. In the native population tuberculosis, protozoan and helminthic infections, pneumonia and malnutrition have commonly been associated with schistosomiasis in endemic areas, while chronic malaria has been prevalent except on Leyte. In China and the Philippines typhoid and paratyphoid fevers are frequently present. In American military personnel the associated infections which have been diagnosed in schistosomiasis cases include: clinical and asymptomatic amebic colitis, amebic hepatitis, bacillary dysentery, relapsed vivax malaria, hookworm disease, strongyloidiasis, ascariasis, infectious hepatitis and pneumonia. The manifestations of these diseases often obscure the picture of schistosomiasis and make accurate clinical diagnosis difficult, if not actually impossible. Thus a tender enlarged liver may be due to amebic colitis or infectious hepatitis. Typhoid fever, acute amebic colitis or bacillary dysentery may produce intestinal discomfort and the latter two diseases provide a stool which may grossly resemble that of the acute stage of schistosomiasis japonica. Chronic malaria has an associated splenomegaly. Pneumonia is at times confused with pulmonary symptoms in schistosomiasis. Moreover, elevation of temperature or change in the blood picture due to the associated disease may so alter these conditions from their characteristic qualities that little or no reliance can be placed on them diagnostically. Finally, light infection of *S. japonicum* in the patient may be completely overshadowed by the temporarily more important one.

#### PROGRESS OF THE DISEASE TO THE CHRONIC STAGE

*General perspective.* The metabolites of the parent worms continue to be discharged into the system for years and are probably primarily responsible for the febrile reaction and the systemic manifestation of intoxication. Yet, more and more, eggs of the parasite come to assume the important rôle in the development of the chronic picture. This is due to the increasing number of eggs which fail to be discharged into the lumen of the bowel but become more or less permanently lodged in the tissues, stimulating a foreign-body reaction, with the end result of miliary pseudo-tubercle formation. This development is much more pronounced in schistosomiasis japonica than in schistosomiasis mansoni because of the much greater fecundity of the female *S. japonicum* and possibly also because of the more abundant secretion of irritating glandular substances by the mature larva of *S. japonicum* inside the trapped egg, which ooze through the shell and provoke very rapid host-cell infiltration. The organs and tissues most seriously involved are the intestine, liver and mesentery.

Healing of the patent hemorrhagic lesions in the intestinal mucosa occurs as fewer eggs escape from the intestinal wall and increasing numbers become trapped in pseudo-tubercles. The wall becomes thickened, fibrosed, loses its tone



and has greatly diminished digestive function. Thus indigestion and dyspepsia increase, and constipation becomes marked, to be interrupted on occasions when dietary indiscretion and physical activity break down the healing process and cause episodes of diarrhea and dysentery. Acute or subacute appendicitis of schistosomiasis etiology is relatively common. Palpation of the abdomen usually demonstrates a thickened, non-resilient colon, while proctoscopic examination frequently reveals cicatricial tissue and papillomata in the sigmoid and rectum. Prolapsus recti may occur and an associated carcinoma of the colon may develop.

Meanwhile an increasing number of eggs are carried in the mesenteric-portal blood into the liver and become lodged in periportal tissues. Within one to two years after exposure the liver begins to shrink, its edges are more rounded and its surface, as indicated by palpation, is studded by multiple minute tubercles. As the liver decreases in size due to the developing fibrosis, the spleen enlarges, until it reaches the umbilicus or at times even to the left of the symphysis pubis. This splenomegaly is due both to inflammation and to engorgement with blood. The increase in mass of the abdominal viscera causes a markedly protuberant abdomen, an elevation of the diaphragm and reduced vital capacity. Infiltration of eggs in the mesentery transforms it into a firm band which at times tends to bind down the abdominal viscera transversely into an upper and a lower half. As a result of the developing periportal cirrhosis, occasionally supplemented by thrombi around worms in the portal vessel, increasing difficulty is experienced by the mesenteric blood in passing through the liver. Sometimes collateral circulation satisfactorily absorbs this burden but usually as the later chronic stage is entered ascites becomes more and more conspicuous. In these patients the superficial abdominal veins become prominent, the abdomen assumes the shape of a greatly distended, inverted gourd, the chest, face and extremities become greatly emaciated, the skin becomes parchment-like and there is a pathetic hepatic facies.

Although the evening elevation of temperature may continue, the blood picture has changed from an eosinophilic leukocytosis to a neutropenia with moderate lymphocytosis and slight eosinophilia. At times there is a marked secondary anemia.

The late chronic stage of schistosomiasis japonica may appear in heavy infections within a year and a half or two years after exposure; more frequently it is observed in four to five years. In native populations in endemic areas it is relatively uncommon to find an individual who has the disease developing from a single inoculation. The average patient gives a history of repeated exposure, so that the symptoms and physical findings are those of early and advanced stages combined.

No infections of schistosomiasis contracted by American or Australian military detachments in the Philippine Islands have had manifestations suggesting that significant chronic lesions had begun to develop, even up to 12 months after exposure. Possibly this is due to the treatment of all active cases of the disease with at least one course of antimony, which has greatly retarded the progress of the pathological process. In the undiagnosed mild or subclinical cases the change

from egg extrusion to tissue repair is gradual and therefore clinically not conspicuous. It is conceivable that in untreated or inadequately treated patients evidences of chronicity will become apparent in five to ten years.

Several hundred cases of chronic schistosomiasis have been observed and several dozen have been examined by the Commission on Schistosomiasis during its stay in the Philippines. Because there are already good descriptions of typical cases of this stage in the Western literature (24, 30, 31), it would be repetitious to give a full clinical account of the cases observed in the Philippines. The report on patients suffering from chronic schistosomiasis will therefore be confined to one family group studied by three members of the Commission (E. C. F., W. H. W., and D. B. McM.) on Mindanao.

#### FAMILIAL SCHISTOSOMIASIS JAPONICA ON MINDANAO, P. I.

This report concerns the development of schistosomiasis japonica in a family group who had lived for one year in a newly discovered endemic focus of the disease, an upland valley at the southwestern base of Mt. Capestrano in north central Mindanao. Before the Japanese invasion in 1942 more than one hundred people had resided in the area. Each family cultivated a small rice and vegetable farm, and possessed a few domestic animals, as chickens, dogs, cats, pigs and carabaos. Running water was used for irrigating the rice fields. Only water from shallow wells was used for bathing and washing of clothes, but children and adults waded in streams to catch fish and snails for food.

The family consisted of eight living members, the father (43 years old), mother (34 years old), twin daughters (13 years old) and four sons (aged 11, 9, 6, and 4 years). The eldest boy had been killed by Japanese snipers two years previously. For a period of thirteen years the family had lived in the town of Malaybalay, some few miles from the disease-producing area, and without exception had been healthy. In January, 1942 they moved to their farm in Barrio Simaya as a means of livelihood and remained there for just one year, when they and their neighbors were driven into the hills by the enemy forces. At the time the group was questioned and examined (July, 1945) they were still refugees in the provincial capital of Valencia. None of them had ventured back to their home because Japanese troops were still in the valley and were resisting Filipino guerrilla forces.

Contact with members of the family was made at the recently rehabilitated Valencia Civilian Hospital, one mile from an American Evacuation Hospital. The mother was assisting as clerk and practical nurse and the children were all within call. The father was working some distance away. All were bright, intelligent Filipinos and spoke English fairly fluently. The twin daughters were quite pretty and very well mannered, and the sons were well behaved. Questioning, physical examination and intradermal tests were conducted at the Civilian Hospital, while blood and stool examinations were carried out at the Evacuation Hospital where better facilities were provided.

Sincere thanks are extended to Col. C. G. Blitch, M.C., U.S.A., Major Claude C. Curtis, M.C., A.U.S., Capt. Ernest B. Freshman, M.C., A.U.S., and Capt.

Colin MacGae, M.C., A.U.S., as well as Tech. Sgt. Ray L. Williams, T3 Don Ludington and T4 Max W. Hornbeak, all of the Evacuation Hospital, and Dr. Apacible, Supt. of the Civilian Hospital, for many courtesies and timely assistance. T3 P. M. Bauman of the Commission assisted in laboratory examinations.

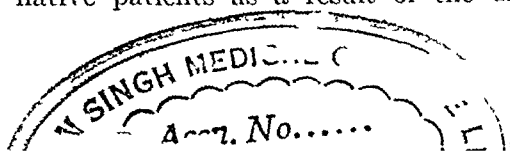
From careful questioning it seems altogether probable that the infection was picked up by repeated small exposures, so that over a period of not more than one year in the endemic area it had developed to appreciable proportions. One of the twin daughters, then ten years old, first became ill about two months after first possible exposure, with dysentery, evening rise in temperature and general malaise. The father and the second daughter followed a few days later and the



MOTHER AND SIX LIVING CHILDREN OF FAMILY STUDIED AT VALENCIA CIVILIAN HOSPITAL  
All members had schistosomiasis japonica

brothers in turn a few months to a year and a half later. All of these individuals had essentially the same type of onset. The mother had evening attacks of fever but never had loose stools. The fever was regarded as tropical malaria but remained untreated because no antimalarial drugs were available. Except for intermittent episodes of dysentery and fever the physical condition of all of the family improved somewhat after they took to hiding in the hills, in spite of the fact that their food was scant and very uncertain.

Many other persons from the same valley had the same disease and some were stated to have succumbed to it. Three members of the Commission on Schistosomiasis (E. C. F., W. H. W. and D. B. McM.), in consultation with medical officers of the Evacuation Hospital, were able to confirm these reports by diagnosis of schistosomiasis in several sick persons who had lived in the same locality. Four deaths of native patients as a result of the disease had occurred in



the Evacuation Hospital from a few weeks to a few days before arrival of the Commission.

#### PROTOCOL OF THE DISEASE IN THE FAMILY

1. *Father*. No physical examination, intradermal test, formol-gel test or personal questioning were possible because he was working at some distance from Valencia. His wife stated that he had the same history of dysentery and fever as his daughters (see below). Thick blood film was negative for malaria parasites. Stool was positive for eggs of *Schistosoma japonicum* and hookworm (direct film).

*Diagnosis*: Relatively mild chronic schistosomiasis and hookworm infection.

2. *Mother*. There was a history of chronic evening chills and fever. She tried to get her daily work done before evening because of this circumstance. She was thin, almost emaciated, but very intelligent and cooperative and was doing everything possible to help her family and her native people.

*Physical examination* revealed abnormalities only in the abdomen: it was slightly enlarged but not protuberant; the parietal tissues were soft and thin, with practically no subcutaneous fat. The liver extended about 2 cms. below the costal margin, was firm and its surface was slightly rough. The spleen was not palpable on deep inspiration. A regional ileitis was clearly demonstrated and the transverse colon was thickened, hardened, tender and movable.

*Blood*: rbc, 4,150,000; Hb, 70; wbc, 9,800; segmented neutrophils 67, stabs 4, eosinophils 9, lymphocytes 16, monocytes 4. Thick blood film negative for malaria parasites.

*Stool*: one specimen was negative for eggs of *S. japonicum*, another positive by direct film. Hookworm eggs were numerous in both films. Intradermal test, +; formol-gel test, +.

*Diagnosis*: Schistosomiasis japonica, mild chronic condition, involving primarily segments of the ileum and the transverse colon, complicated by moderately severe hookworm infection.

3. *Rebecca* (13 yrs. old). She was the first member of the family to develop clinical manifestations, with a discharge of bloody mucus and at times pure blood in the stool. About a year ago she had evening fever. Dysentery still occurs periodically, and late afternoon fever persists. She has a poor appetite. She does household work, as cooking and washing, although she tires easily. Childhood diseases included only measles and chickenpox.

*Physical examination*: A bright, pretty, very well mannered, fairly well nourished adolescent; she is about 54 inches tall and weighs about 65 pounds. Her mouth and teeth are in splendid condition. All superficial lymph glands are normal in size and consistency. The muscle tone is good. Pulse, 78; good heart tone, but with accentuated P<sub>2</sub>; lungs clear. There is a prominent left upper abdominal quadrant (fig. 1) due to a large, firm, tender spleen, which is very painful to palpation and extends to one cm. below the left iliac crest and posteriorly. The liver extends 3.5 cm. below the costal margin; it is very tender, but not especially firm; the caudate lobe is not very prominent. The abdomen is soft, only slightly protuberant and gives a feeling of primary peritonitis.

*Blood:* rbc, 4,700,000; Hb 85; wbc, 14,900; segmented neutrophils 56, stabs 3, eosinophils 7, lymphocytes 31, monocytes 3. Thick blood film negative for malaria parasites. Intradermal test, +; formol-gel test, + + + +.

*Stool:* positive for eggs of *S. japonicum* and hookworm (direct film).

*Diagnosis:* Severe early chronic schistosomiasis japonica, complicated with hookworm infection.

4. *Cleofa* (13 yrs.). She developed clinical manifestations, including chills and evening fever, with blood and mucus in her stool, at the same time as her father and shortly after her twin sister became ill. At the present time she states that she does not feel particularly sick but is not strong, tires easily and has an evening fever. As a small child she had measles, chickenpox and whooping cough.

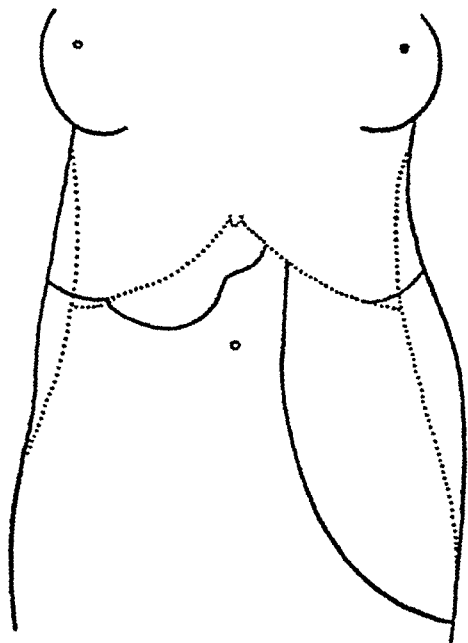


FIG. 1

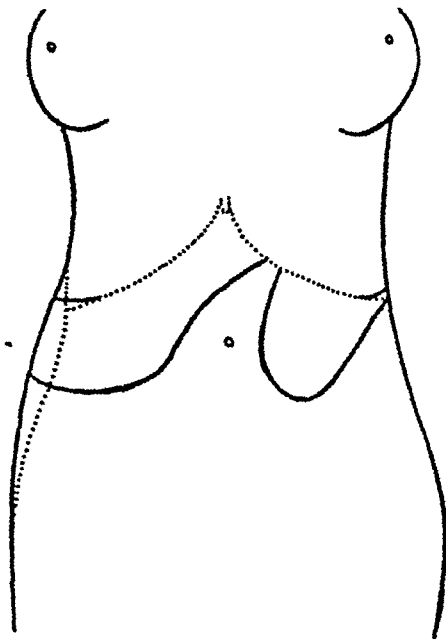


FIG. 2

FIGS. 1 AND 2. OUTLINE OF BODY, COSTAL MARGINS AND SUBCOSTAL LIMITS OF LIVER AND SPLEEN IN TWIN SISTERS OF SCHISTOSOMIASIS FAMILY (Fig. 1, Rebecca; fig. 2, Cleofa.) Differences between solid and dotted lines indicate abnormal changes in contour

*Physical examination:* A bright, almost beautiful girl, well nourished; is starting to mature and has the typical adolescent reaction to physical contact; she is about 53 inches tall and weighs about 63 pounds. Her mouth and teeth are in splendid condition. All superficial lymph glands are normal in size and consistency. The muscle tone is good. Pulse 77; good heart tone; lungs clear. The liver is enlarged to 2 cm. below the umbilicus (fig. 2), is smooth, with a sharp edge, not tender on palpation. The spleen is enlarged 5.5 cm. below the costal margin, has a rather soft consistency, and is painful. The abdomen is soft.

*Blood:* rbc, 4,280,000; Hb, 80; wbc, 14,200; segmenting neutrophils 5, stabs 4, eosinophils 9, lymphocytes 32, monocytes 4. Thick blood film negative for malaria parasites. Intradermal test, negative; formol-gel test, + + + +.

*Stool:* positive for eggs of *S. japonicum* and hookworm (direct film)

eosinophils 15, lymphocytes 32, monocytes 2. Thick blood films negative for malaria parasites. Intradermal test, +; formol-gel test,  $\pm$ .

*Stool*: Positive for eggs of *S. japonicum* and hookworm (direct film).

*Diagnosis*: Moderately severe early chronic stage of schistosomiasis japonica, complicated with hookworm infection and malnutrition.

8. *Rogelio* (4 yrs.). He became noticeably ill about one year ago, the last of the family to develop the characteristic evening fever, diarrhea and dysentery. There has been a periodic repetition of these episodes about every two weeks. At present he is relatively free of symptoms, has a good appetite and plays with considerable energy.

*Physical examination*: He is well nourished, cheerful and very active; he is about 36 inches tall and weighs about 32 pounds. The head and neck show nor-

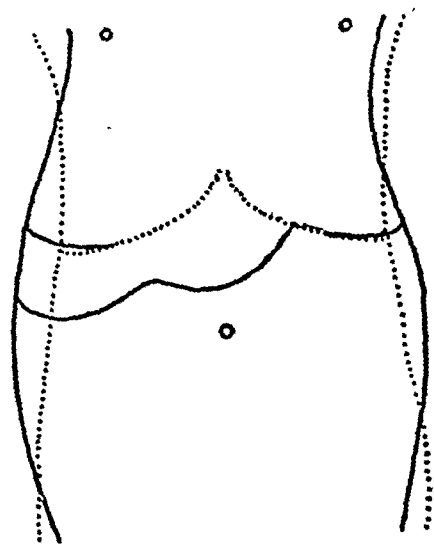


FIG. 5

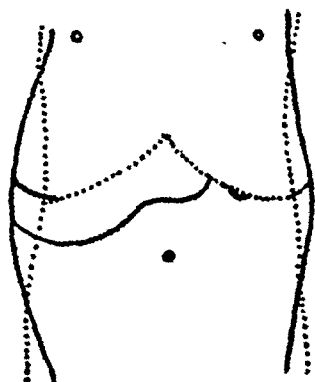


FIG. 6

FIGS. 5 AND 6. EXPLANATION AS IN FIGS. 1 AND 2  
(Fig. 5, Camilio; fig. 6, Rogelio)

mal relations. The mouth is normal. There is no nuchal rigidity. Pulse 72; heart sounds of good quality; lungs clear. The superficial lymph glands are normal except both right and left epitrochlears, which are enlarged. The abdomen is not protuberant. The liver (fig. 6) is enlarged about 2.5 cm. below the costal margin and has a distinct caudate lobe; it extends upwards to the sixth anterior interspace; it is smooth, fairly firm, with slightly rounded edges. The spleen is barely palpable on deep inspiration.

*Blood*: rbc, 4,450,000; Hb 70; wbc, 15,300; segmenting neutrophils 43, stabs 5, eosinophils 17, lymphocytes 32, monocytes 3. Thick blood film negative for malaria. Intradermal test, negative; formol-gel,  $\pm$ .

*Stool*: Positive for eggs of *S. japonicum* and hookworm (direct film).

*Diagnosis*: Active, rather mild, late acute or incipient chronic stage of schistosomiasis japonica, complicated with hookworm infection.

## ADDITIONAL CASES FROM THE SAME LOCALITY

This group includes a young man of 17 years, with physical findings of mesenteric and peritoneal involvement suggesting peritoneal tuberculosis, a very tender, enlarged, nodular liver, a hard palpable spleen, ascites and prominent superficial abdominal veins; three children of another family, including a boy of 10 years with severe late chronic infection, a girl of 8½ years with moderately severe early chronic infection, and a girl of 7 years with light chronic infection; and a small boy (2 years old with the development of an 8-months-old infant) exhibiting a fairly advanced severe infection. The mother of the small child states that other children of her family have the same disease. All of the individuals examined were demonstrated by stool examination to be infected with *S. japonicum* and hookworm.

## DISCUSSION OF THE FAMILIAL INFECTIONS

The family of eight were all infected and had acquired the disease within the limits of one year (1942). Had they remained in the town of Malaybalay, where they had resided for seventeen years previously, they would undoubtedly have escaped the infection. Had they lived longer on their farm, they would have been subjected to additional exposure and would in all probability have exhibited more severe symptoms, with an acute state of the disease superimposed on the chronic condition of the disease previously contracted.

The sequence of onset in the family is interesting and instructive. The father and twin sisters came down first, followed in succession by the four boys in the order of their ages. The youngest boy was only 2 years old when the family escaped to the hills, and had been subject to exposure for only a short time. The eleven- and nine-year-old boys have the most severe infection in the family. The girl, Cleofa, who is less active than her sister, was possibly exposed only once and has only a slightly more advanced state of the disease than her four-year-old brother. The history of the mother's exposure is uncertain: she possibly had light exposure on several occasions. The development of her infection was apparently insidious, and her abdominal symptoms are no doubt due to hookworm as well as to *S. japonicum* lesions.

In contrast to this large family group is the 17-year-old boy with advanced chronic schistosomiasis. While all of the six children in the infected family have a poor ultimate prognosis if they remain untreated, the advanced portal cirrhosis in the one boy indicates that he is already beyond the stage of therapeutic help. He is typical of the children in endemic areas, who begin to contract the infection early in life and by repeated exposure soon acquire an amount sufficient to produce profound, irreparable damage to the liver.

No one of the native cases on Mindanao, and no other Filipino seen by the Commission, exhibited nuchal tenderness or rigidity, which were conspicuous manifestations of the acute infection in American military personnel. Moreover, except for the youngest boy in the family group, none of the infected persons exhibited a significant eosinophilia at the time of examination and even in his

case there was a neutropenia, suggesting that the disease had possibly passed its acute stage. In general, it is notable that no member of this family had a history of severe, prostrating onset, as was experienced by so large a percentage of the hospitalized American military cases.

The formol-gel test provides some evidence of the chronicity of the disease. In the two girls and the two eldest boys the test was strongly positive; in the mother and two youngest boys it was  $\pm$ . Except for the possible exception of the mother this corresponds to the history of exposure and the physical findings, since early cases of infection typically give a weak or negative formol-gel test.

#### DIFFERENTIAL DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

As has been indicated in earlier portions of this study, schistosomiasis japonica has three separate consecutive stages in its development, the incubation period, acute stage and chronic stage. Each stage has relatively reliable diagnostic landmarks, provided the physician is conscious of the possible occurrence of the disease in his patients and recognizes the characteristic features at each stage in its development.

*Incubation period.* During the developmental stage there are few trustworthy symptoms and findings until towards the latter part of the period. The prickling dermatitis which develops in some exposed individuals a few hours after contact with infested water is not easily differentiated from a dozen other types of transient dermatitis which are so prevalent in endemic areas. The pulmonary involvement may escape notice entirely or be regarded as a "bronchial" cold. The development of urticaria may be a manifestation of food sensitization, or may be diagnosed as a form of angioneurotic edema. The malaise and daily fever may suggest undulant fever, chronic falciparum malaria or a mild toxic state of undetermined etiology. The abdominal fullness and epigastric distress may be tentatively regarded as incipient typhoid fever, the hepatic enlargement and tenderness as infectious hepatitis, leptospirosis or amebic hepatitis, and the prodromal mucous diarrhea as early amebic colitis. Only when the temperature curve is studied and found to have a consistent rise each evening and the blood picture indicates a significant eosinophilic leukocytosis can most of these possible etiologies be eliminated as the primary or exclusive cause of the illness.

Nevertheless, experience has shown that pneumonia, pulmonary tuberculosis, single or multiple intestinal helminthiasis, amebic colitis or hepatitis, bacillary dysentery, typhoid fever, undulant fever, infectious hepatitis, malaria, and other infectious diseases are frequently associated with schistosomiasis japonica, so so that these complications at times obscure the developing syndrome. A clear history of wading, swimming or otherwise exposing the skin or mucous membranes to untreated water in known endemic areas strengthens the suspicion, but malaise, abdominal distress, diarrhea and eosinophilia have been used as a basis for clinical diagnosis when the subsequent history of the patient fails to support this view. Specific confirmation of a tentative clinical diagnosis can be obtained only when eggs of the parasite are discharged by the female worm and are evacuated in the stool. This comes at the end of the incubation period.



*The acute stage.* In this stage the dominating symptoms and findings are abdominal. They frequently involve fullness and tenderness of the entire abdomen and may suggest an acute abdomen or peritonitis. At times there is an appendiceal syndrome; again it may be an ileitis or involvement of the transverse colon which predominates. Usually there is an accompanying enlargement of the liver, extending below the costal margin and upwards to cause the diaphragm to bulge into the pleural cavity. There is usually tenderness in the right hypochondriac region and acute pain on palpation or percussion of the liver. Amebic colitis with hepatic extension is a reasonable diagnosis which might be made at this time but, as in the late stage of the incubation period, a study of the fever chart and the blood picture indicates that this is not tenable. Moreover, in any patient who is moderately to severely ill during this stage, eggs can usually be recovered without difficulty in the bloody-mucus portion of the stool, thus clinching the diagnosis. Finally, proctoscopic examination of a patient with uncomplicated schistosomiasis japonica will show that the lower sigmoid colon and rectum never show the lesions indicative of amebic or bacillary colitis but minute yellowish nodules or injected capillaries, samplings from which will usually provide demonstration of typical *Schistosoma japonicum* eggs. A positive intradermal test is also relatively confirmatory in the presence of the characteristic clinical findings.

*The chronic stage.* If this stage is uncomplicated either by an earlier stage of schistosomiasis or by a disease of other etiology, there is clear evidence of a progressive fibrosis of the bowel together with periportal cirrhosis. At first the liver is enlarged and remains tender, due to continued inflammation, but gradually it becomes fibrosed, with miliary pseudo-tubercles studding its surface, and begins to shrink. Reciprocally, the spleen enlarges as a result of congestion and the eventual fibrosis of its trabeculae. As a result of the fibrosis or thrombosis in the portal vessel ascites develops and the superficial abdominal veins become distended. The mesentery becomes involved in the fibrotic process and may bind down the abdominal viscera. Thus, the picture during this period is one of rapidly or gradually developing fibrosis of the intestine, liver and mesentery.

The daily rise in temperature in the evening tends to persist and there is usually a slight eosinophilia, although the total white blood cells are reduced and the lymphocytes relatively increased.

Whatever the other symptoms and findings may be, those due to hepatic cirrhosis are predominant. Thus, the general diagnosis is settled but the etiology must be discovered. In most instances the eggs of *S. japonicum* can be recovered in the stool, at times only after sedimentation or other good concentration technics. Occasionally biopsy of papillomatous growth in the rectum will provide the specific evidence when several stool specimens have been negative. The formol-gel test is typically positive. The only other disease in which this test is consistently positive is kala-azar, a disease in which there are hepatomegaly and splenomegaly but without portal cirrhosis and ascites.

*Complications.* These may occur at any stage in schistosomiasis japonica. The most confusing complications resulting from the disease itself are due to the

escape of eggs during the acute stage and their deposition in foci far removed from the abdominal viscera. Thus, pulmonary, end-arteriolar, cutaneous and neurological manifestations must be considered as possible extensions of the disease when schistosomiasis japonica exists.

#### SUMMARY

Schistosomiasis japonica is a disease which has been known clinically for a century and its etiology has been understood for nearly half a century. Its present importance has resulted from exposure of several hundred American and Australian troops on Leyte, Philippine Islands from October 20, 1944 through the winter and early spring of 1945.

Three stages of the disease are recognized, incubation, acute and chronic. There has been a paucity of observations on patients during the incubation period because in native populations in endemic areas the infected individuals seldom see physicians until the disease is well advanced. Each of the three stages is considered; native and military case histories are utilized to provide a relatively full picture of the clinical progress of the disease from the time of exposure until the late chronic condition has been attained. A special report is included on this infection in a family group of eight, from a newly discovered endemic focus on Mindanao, P. I.

The differential diagnosis of schistosomiasis japonica is discussed for each of its three stages of development. While there are certain relatively characteristic symptoms, signs and physical findings at each stage of the disease, it is frequently hazardous to depend on these findings alone. Recovery of the eggs of the etiological agent, *Schistosoma japonicum*, constitutes the only method known of specific diagnosis. Since the eggs are not deposited until the worms mature, only tentative diagnosis can be made during the incubation period. They are easily recovered in moderate to heavy infections during the acute stage, but as the chronic stage progresses they are discharged into the lumen of the bowel in increasingly smaller numbers. During this later period considerable diligence may be required to obtain egg confirmation of the clinical diagnosis. The intradermal test or formol-gel test add strength to a presumptive diagnosis.

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# THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

## II. THE DIAGNOSTIC CHARACTERISTICS OF THE EGGS OF THE ETIOLOGIC AGENT *SCHISTOSOMA JAPONICUM*

ERNEST CARROLL FAUST<sup>1</sup>

### INTRODUCTION

In a previous communication from the Commission on Schistosomiasis (1) information has been presented on the symptoms, signs and physical findings characteristic of schistosomiasis japonica at different stages in the development of the disease. The present paper deals with the characteristics of the eggs of *S. japonicum* obtained from human and animal hosts. The importance of the subject is indicated by the fact that while typical, viable, mature eggs of this parasite are readily diagnosed by clinical laboratory workers once they have seen the eggs, immature and degenerate eggs are not always recognized as belonging to this parasite and plant-cell artefacts in feces are at times mistaken for "atypical eggs".

The adult *Schistosoma japonicum* lives in the mesenteric-portal venous circulation, very frequently in the mesenteric venules within the muscular and mucous layers of the small intestine, cecum, appendix and colon. In these locations the delicate female is held in position in the venule by the stouter male, with her anterior end directed towards the mesenteric capillary. She lays one egg after another in advance of her head until an entire venule is filled with eggs. Then the pair of worms back up and advance into an adjacent venule, in which oviposition is repeated. Thus, in a period of days or weeks all of the small venules in the vicinity of a mated pair may become packed with eggs.

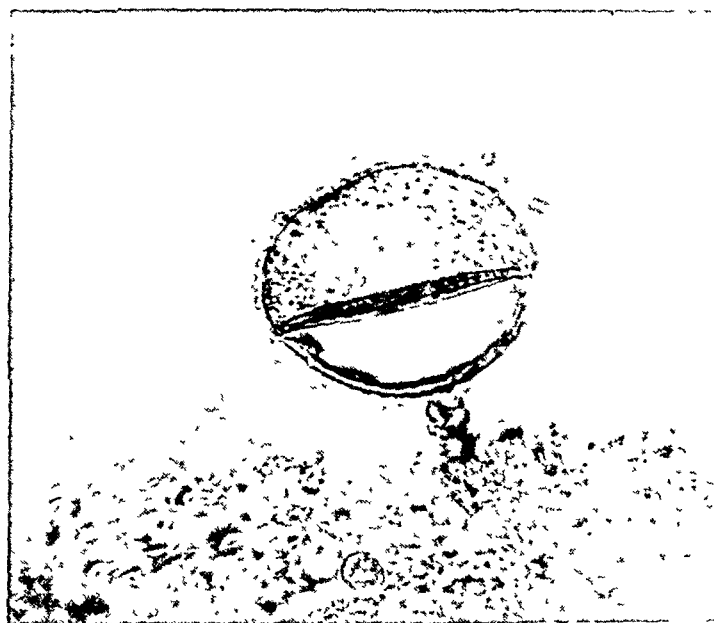
Even though the newly deposited eggs are immature, they are considerably larger than the normal diameter of the venule, causing it to distend where each egg is laid but with constriction of the vessel between each two eggs. In this way the flow from the mesenteric arterioles through the capillaries is blocked. Once deposited, the majority of the eggs mature rapidly and the larva (miracidium) inside each egg secretes cytolytic fluids which ooze out through submicroscopic pores in the egg-shell and cause the delicate wall of the venule to be weakened. The back pressure in the blood vessel, the lytic action of the fluid secreted by the maturing larva and the peristaltic movement of the bowel combine to cause rupture of the blood vessel and filtration of the eggs, together with extravasated blood, through the submucous and mucous coats of the bowel into the lumen. The mature eggs, less frequently the immature ones, are carried down the bowel and are evacuated in the stool.

Little by little, as the female worms continue to oviposit into mesenteric ven-

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ules and the eggs escape from the veins, tissue reaction develops around each egg in the form of a pseudo-abscess. If the pseudo-abscesses are near the lumen of the bowel they may be expelled *in toto*, or a portion of the adventitia may be discharged with an egg.

At times immature or mature eggs degenerate, and even become calcified before they are evacuated from the tissues. Moreover, more deeply buried pseudo-abscesses proceed to pseudo-tubercle formation and some of these occasionally are expelled from the surrounding tissues. Finally, in patients undergoing antimony treatment, the eggs in the perivascular tissues and those within the venules gradually undergo degenerative changes, so that they become increasingly atypical in appearance.



PHOTOMICROGRAPH 1. SHELL OF *SCHISTOSOMA JAPONICUM* EGG FROM WHICH MIRACIDIUM HAS RECENTLY HATCHED

Recovered from sedimented feces ten hours after sedimentation had been completed. Free-swimming miracidia were abundant in the top of the supernatant water.  $\times 350$ .

Thus, accurate diagnosis of the eggs of *Schistosoma japonicum*, like those of the related species *S. mansoni* and *S. haematobium*, requires recognition not only of the typical mature eggs but also of the immature and degenerate types.

In this paper representatives of these several stages are described and illustrated. All observations have been made first on living specimens, checked at times by intra-vital cresyl blue staining and formalin-preserved material.

#### VIABLE EGGS

*Immature eggs.* These eggs are commonly found within the mesenteric venule and in lesser numbers filtering through the perivascular tissues, but in case extensive hemorrhage from the blood vessels occurs they are discharged into the intestinal lumen and appear in the stool.

The earliest stage observed in hosts' excreta (fig. 1) was obtained from the bloody mucus of a heavily infected dog. It is biconvex like the shell of a rather flat clam, measures 58 by 44 microns and has a noticeable wart-like thickening near one end at the site where an abbreviated spine is sometimes seen. It is slightly asymmetrical. Internally there is a delicately granular, flattened, fertilized ovum, surrounded by more heavily granular, denser yolk material which extends to the inner margin of the shell. The next stage, which was found in the stool of the same host (fig. 2), is about the same size (60 by 48 microns) but is more symmetrically oval. The egg cell is more nearly rotund but the first cleavage has not yet taken place.

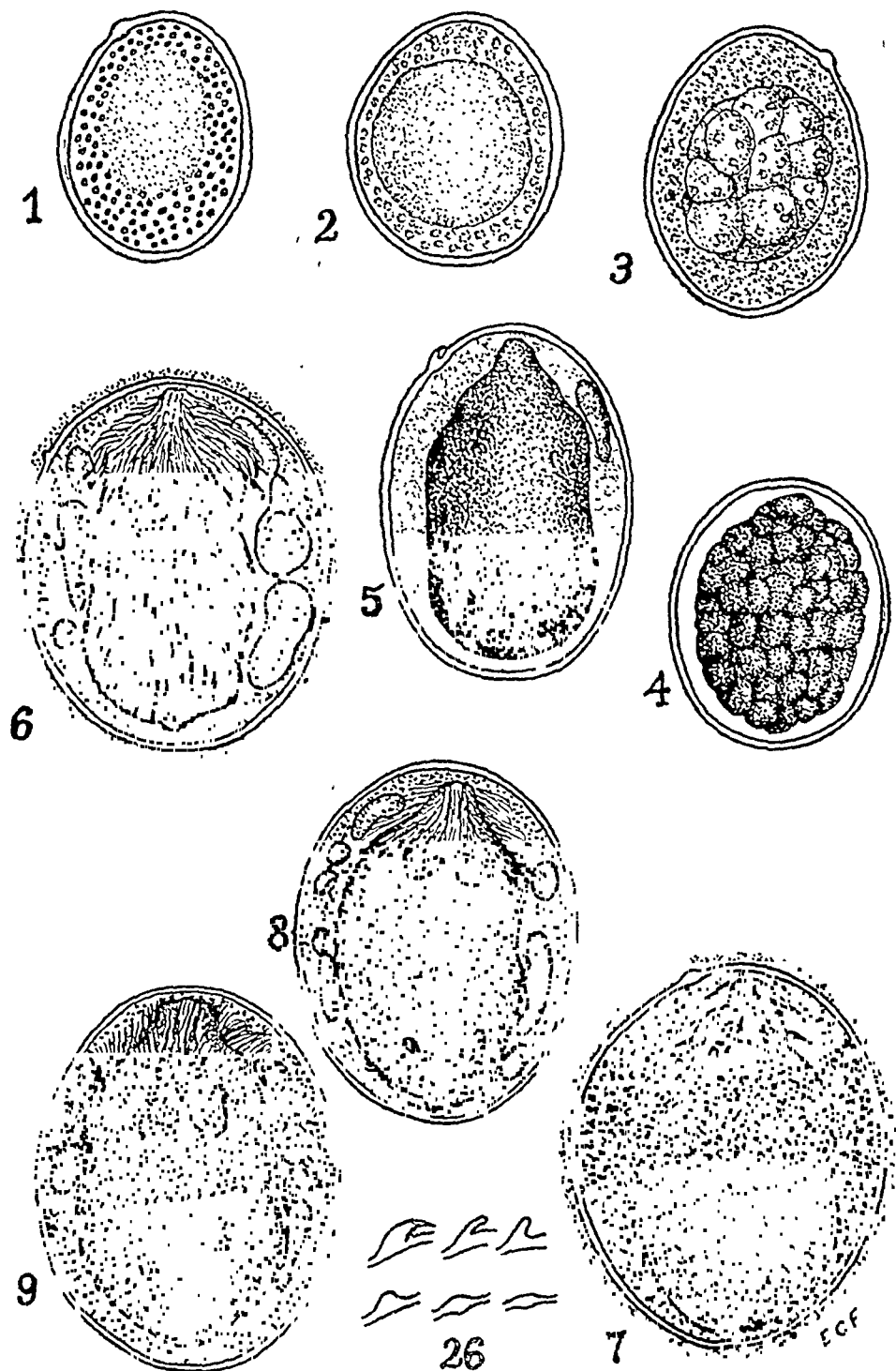
Distinct internal development is noted in Figure 3. Here the egg is appreciably larger (70 by 52 microns), is beginning to assume a definite roundness in transverse diameter and has an embryo with approximately sixteen blastomeres. Moreover, the yolk material is beginning to disintegrate as the embryo makes use of the stored-up nourishment. The shell is still comparatively unstretched and has a definite lateral wart-like thickening at one end. Although the egg pictured in Figure 4 is slightly smaller than that in Figure 3 (66 by 50 microns), there are three indications of additional development, namely the embryo is now an advanced gastrula-like organism, the yolk material has entirely disappeared and the shell is appreciably thinner.

In Figure 5 the embryo inside the shell is beginning to assume the contour of the mature larva, although cilia and internal organs have not yet appeared. The length is approximately that of a mature egg (86 microns) even though the transverse diameter is still comparatively rather narrow, and metabolites of the rapidly growing embryo are accumulating as minute granules and semi-translucent masses between the embryo and the shell.

*Mature eggs.* Figures 6 to 13 illustrate typical mature eggs, with distinctly visible cilia which are frequently in motion. These eggs vary in size from 86 x 70 microns (fig. 8) to 97 x 72 (fig. 9), 99 x 78 (fig. 7) and 82 x 80 microns (fig. 11), depending in part on the intrinsic size of the particular egg and in part on the stretching of the shell that has occurred as a result of the activity of the fully developed motile larva within. Emphasis has been placed on the intrinsic size of the egg, since fully mature eggs with motile larvae (miracidia) have been observed which are no larger than half-developed eggs (see fig. 4), and others have been measured which have giant proportions (i.e., 150 x 122 microns). Viable eggs ready to hatch are typically broadly ovoidal (figs. 6-10) but they may be almost spherical (fig. 11).

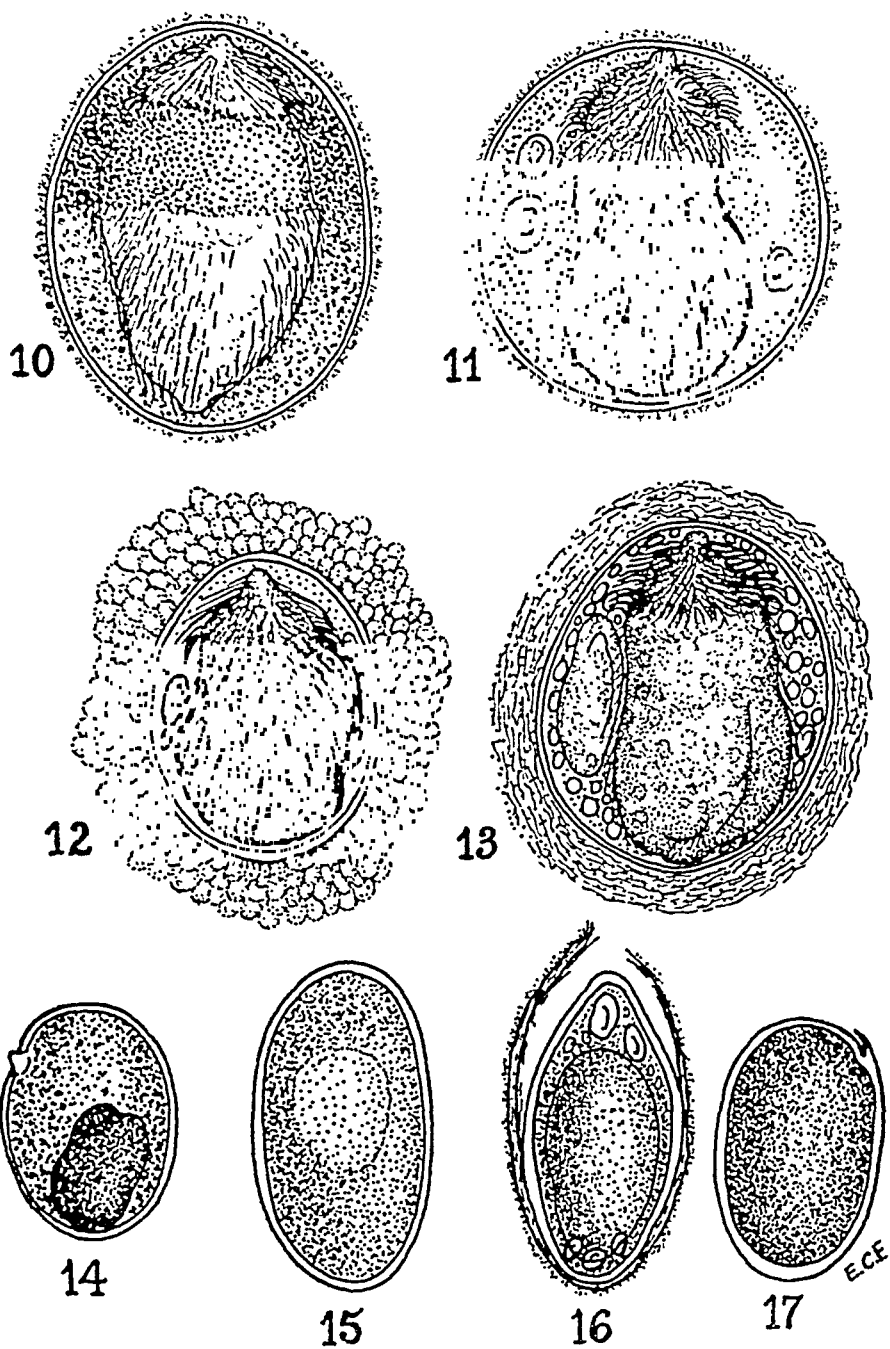
At times the only visible activity of the mature viable miracidium is the beating of the cilia of the four flame cells within its body (figs. 8, 9, 11); at other times there may be energetic wave-like action of the cilia which cover the larva. Again, the larva may be nervously squirming about inside the shell, in an attempt to escape from its prison. Occasionally it turns on itself and completely reverses its axis within the shell, although this is not so easy to detect as it is in *Schistosoma mansoni*, in which the distinct lateral spine serves as a directional landmark.

In the mature stage of the egg it is frequently possible to observe two types of internal glands, both apparently digestive in function. The one type consists of

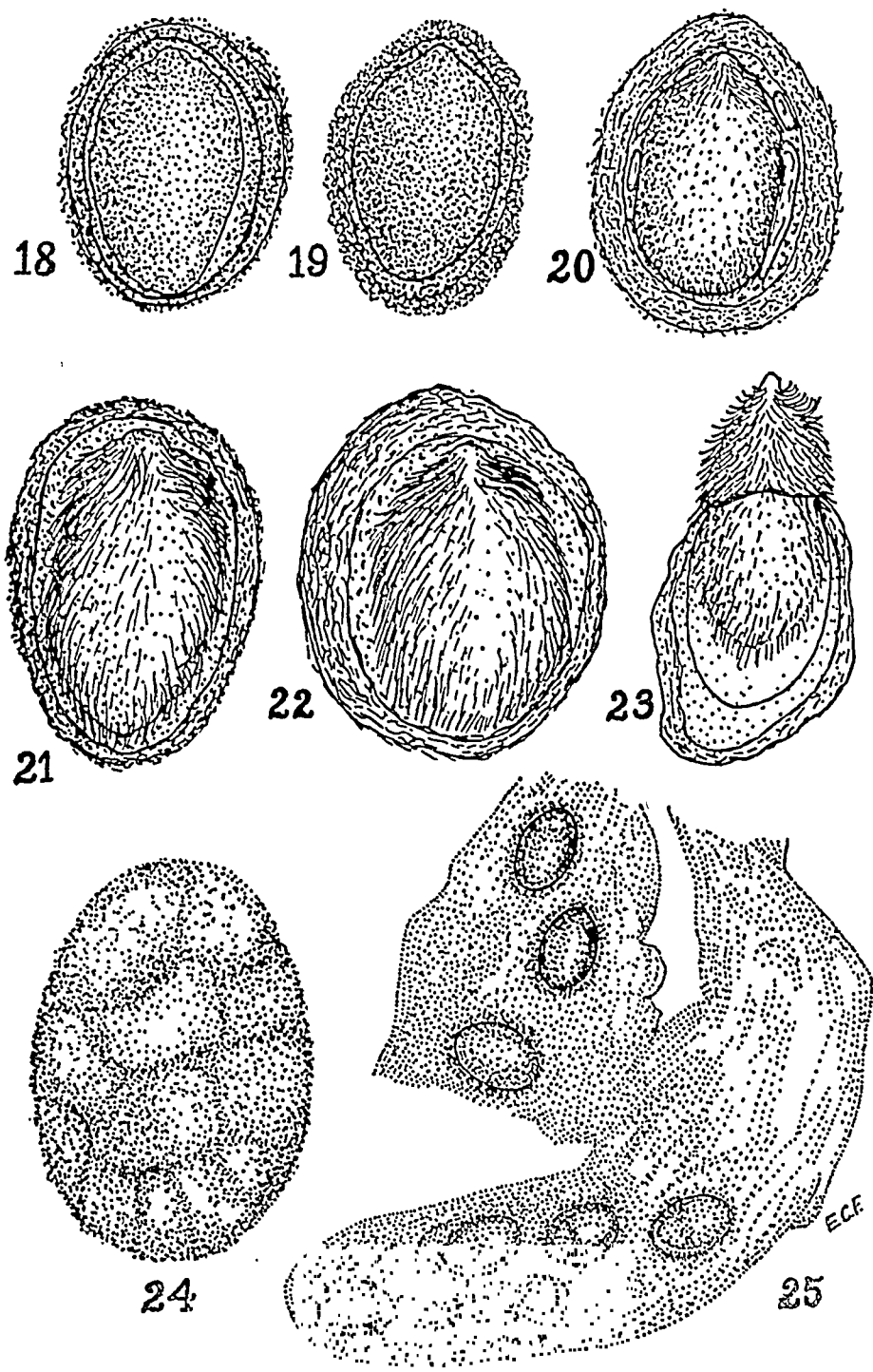


FIGS. 1-9 AND FIG. 26





FIGS. 10-17



FIGS. 18-25

two distinct unicellular glands (figs. 8, 9), with finely granular contents, which discharge their secretions on either side of the primitive gut in the anterior papilla. The other type consists of a mass of several smaller glands, median and somewhat more posterior in position, with semi-opaque contents, which discharge their mucous secretions laterally at about the junction of the anterior and middle thirds of the body (fig. 6). Spherical and elongate masses of these tend to accumulate between the larva and the shell (figs. 6, 8, 9, 11). Since the mucoid secretions stain a delicate pink with eosin and similar pink radiations extend out from the shell in hematoxylin-eosin-stained fixed tissues in which eggs have been trapped, it seems possible, although not directly proven, that it is the mucoid secretions which digest the tissues through which the eggs filter out of the mesenteric-portal veins.

In some mature viable eggs there is a distinct, broad band of granules within the shell, which encircle the miracidium just back of the openings of the mucous-secreting glands, and hence just behind the last row of curved body cilia (figs. 7, 9, 10). These bands appear dark brown to blackish in transmitted light under the compound microscope, probably due to the opacity of the granules of which the bands are composed. Their origin is not clear, but they are not present in somewhat immature eggs (fig. 5) or in those having larvae with distinct cilia which are not yet motile (fig. 6).

In unhatched viable mature eggs it is not usually possible to distinguish the embryonic membrane that envelopes the larva up to the time it escapes from the shell and becomes a free-swimming organism.

Most of the eggs containing active larvae (figs. 6, 7, 10, 11) have an agglomerated coating of neutrophilic and eosinophilic leukocytes, necrotic tissue cells, granules and minute globules. This is interpreted as being due to the secretions of the miracidia which ooze out through minute pores in the shell, temporarily "glue" small cellular elements to the shell and perhaps attract phagocytic cells. This adventitious coating is rather characteristic of the mature, viable egg which is evacuated in the stool. Shaking of such eggs vigorously in a 5 per cent solution of sodium sulphate dissolves the surrounding layer of cells and leaves the shell smooth and glistening.

Occasionally an egg with motile miracidium will be discharged from the in-

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FIGS. 1-4. Successive early stages in the development of the egg of *S. japonicum*; figs. 1 and 2 from dog's feces, figs. 3 and 4 from human feces.  $\times 500$ .

FIG. 5. Slightly immature egg of *S. japonicum*, from human feces.  $\times 500$ .

FIGS. 6-13. Mature, viable eggs of *S. japonicum*; figs. 6, 7, 10 and 11 from human stool, figs. 8, 9, 13 from dog's stool.  $\times 500$ . Note: figs. 7, 9 and 10 each have transverse bands of semi-opaque granules surrounding the second fifth of the miracidium; the egg in fig. 12 is encased in a pseudo-abscess, that in fig. 13 in a pseudo-tubercle.

FIGS. 14-23. Different types and stages of degenerate and dead eggs of *S. japonicum*.  $\times 500$ . Figs. 14, 15 and 17, degenerate eggs which have never started to mature; figs. 16, 18, 19, 20, half mature to nearly mature dead eggs on the thirteenth day of fuadin treatment; figs. 21-23, dead mature eggs on the thirteenth day of fuadin treatment.

FIG. 24. Mature, completely calcified egg of *S. japonicum* from human stool.  $\times 500$ .

FIG. 25. Small fleck of intestinal mucus from man containing several trapped eggs of *S. japonicum*.  $\times 125$ .

FIG. 26. Representative spines and protuberances from eggs of *S. japonicum*, recovered from human and dog's stools, Leyte, P. I.  $\times$  ca 1000.

testinal wall within a thick pseudo-abscess (fig. 12) or even pseudo-tubercle (fig. 13), which has been forced *in toto* out of the tissues. The structure appears as an unhusked walnut or coconut shell and has an overall measurement considerably in excess of the mature egg.

#### DEGENERATE EGGS

*Immature eggs.* In the stools of both human and dog hosts many types and stages of degenerate immature eggs of *S. japonicum* have been observed, from early fertile but unsegmented stages (figs. 14, 15) to dead eggs which have partially or almost completely developed (figs. 16, 18, 19, 20). Similarly, several types of degenerate infertile eggs have been observed, of which figure 17 is rather typical. The degenerate eggs may be partially calcified (fig. 14) or completely calcified (fig. 17), either naked when found in the stool or enveloped in a relatively thick tissue capsule (figs. 16, 18, 19).

*Mature eggs.* Mature eggs are frequently killed as a result of their entrapment in pseudo-tubercles. They may show the normal contour of the miracidium, and even still possess distinct cilia (figs. 20, 21, 22); or they may have become completely calcified (fig. 24) so that they retain only the outline of the shell as the one identifying character. These stages are particularly difficult to identify, yet they have been observed in considerable numbers, with and without association with normal mature eggs, in the stools of patients having chronic schistosomiasis. All of these stages, as well as "still-born" miracidia (fig. 23), have also been found in stools of infected patients undergoing antimony treatment.

#### NESTS OF MUCUS-TRAPPED EGGS

In stools containing flecks or shreds of mucus, with or without macroscopic evidence of blood, eggs of *Schistosoma japonicum* may be trapped in considerable numbers (fig. 25). They may be so masked by undigested plant cell elements which are also entangled in the mucus that accurate identification of the eggs is difficult. Moreover, in such instances there may be very few eggs in the fecal portion of the stool, so that it is particularly desirable to free the suspected eggs from the mucus in order to facilitate diagnosis. This can usually be accomplished by vigorously shaking the mucus elements of the stool in a test tube with a 5 per cent solution of sodium sulphate, which will separate the eggs from the mucus without damaging their diagnostic characteristics.

#### EGGS IN INTESTINAL "NODULES" AND IN DILATED INTESTINAL CAPILLARIES

While careful search of the stools of infected persons will usually be rewarded by the recovery of diagnosable eggs of *S. japonicum*, particularly if concentration technics are employed, there is the occasional case with negative stools in which proctoscopy reveals minute pinpoint nodules, which are creamy or light yellowish in color in contrast to the normal pink of the intestinal mucosa. Even during the acute stage of schistosomiasis japonica one or more of these nodules may be visualized just above the sigmoido-rectal junction or immediately below that level. Biopsy of this nodule reveals a rather hard nucleus in which several eggs

of *S. japonicum* are nested. These nodules are seen proctoscopically in a certain proportion of individuals whose stools are positive for *S. japonicum* eggs, but their particular diagnostic usefulness is in the patient whose stools have been consistently negative.

In *S. japonicum* patients having no proctoscopic evidence of so-called "typical yellow nodules" localized or generalized dilation of the intestinal capillaries may sometimes be seen, with a diffuse hyperemia of the lower colonic and rectal mucosa. An attempt at aspiration will usually produce rupture of the wall of the capillary and the microscopic specimen of blood in the aspirate will possibly contain one to several viable eggs. These may be immature but more frequently contain motile miracidia.

#### EGG SHELLS

When sedimentation or other egg-hatching technic has been employed to obtain free-swimming miracida of *S. japonicum*, microscopic films of the sediment contain typical egg-shells, with a slit on one side, through which the miracidium has escaped. These shells are diagnostically useful and in the absence of whole eggs may be relied on for accurate diagnosis of the infection (see photomicrograph 1).

#### SHELL SPINE OR PROTUBERANCE

*In immature eggs.* Frequently, although not invariably, there is an abbreviated spine or a warty thickening of the shell of *S. japonicum* on one side near the anterior end. If it is present, it is readily observed in distinctly immature eggs (figs. 1, 3), since they are biconvex in a dorso-ventrol position. The protuberance may be a sharp incurved spine (figs. 5, 17), an awkwardly projecting minute papilla (fig. 14) or merely a slightly raised mound-like thickening at the site (fig. 7). Examination of tens of thousands of immature eggs from *S. japonicum* infections in the Philippines has demonstrated that the protuberance is even less frequently noted in this endemic area than in China and Japan.

*In mature eggs.* As the shell of the maturing egg swells and rounds out its contour, any spine-like or warty protuberance usually becomes stretched out and less evident. Moreover, the shell is much less likely to lie on its ventral or dorsal side, so that any thickening is apt to be concealed unless the egg is carefully rotated on its longitudinal axis to its dorso-ventral position (see fig. 6).

Following a concentration technic employing sodium sulphate, the accumulation of cellular debris around the egg-shell of *S. japonicum* is removed, the shell contour becomes smooth as glass and any thickening is much more readily discovered.

#### DISCUSSION

This presentation consists of a description of a relatively complete series of eggs of *Schistosoma japonicum* recovered from the stools of the two most commonly infected hosts, man and dog. The viewpoint particularly emphasized is the wide range of sizes, shapes, stages of immaturity or maturity, viability or

degeneration which the laboratory diagnostician is likely to encounter in examining stools of persons harboring this parasite. Previous students of *Schistosoma japonica* have described the stages of development from fertile egg *in utero* to the viable ones containing motile miracidie (Faust and Meleney, 1924) (2), or have utilized as the material for study a series of eggs obtained by scrapings of the intestinal mucosa of experimentally infected mice, rabbits, guinea pigs, dogs and monkeys (Vogel, 1942) (3). While an attempt has been made in the present paper to provide accurate morphological information both in the text and in the illustrations, details of internal structure of the developing or mature miracidium have been included only in so far as they can be readily observed by the average clinical laboratory worker who has had sufficient microscopic training and experience to appreciate the differences between immature and mature stages of development, and living, dying or degenerate specimens.

Accurate diagnosis of the eggs of *Schistosoma japonicum* constitutes the only known absolute confirmation of the clinical findings in the infection. Recognition of immature eggs may allow specific treatment to be undertaken from one to several days before typical mature eggs appear in the stool (Vogel, 1942) (3). Again, the lightly infected person may be asymptomatic or asyndromic and laboratory diagnosis from routine stool examination may constitute the sole method by which the infection is detected.

In addition to the diagnostic importance *per se* of the accurate recognition of the eggs of this parasite, there is considerable prognostic significance attached to the exact stage of the egg recovered from the stool. This is well demonstrated in some of the eggs described and illustrated in this paper. One example will be cited.

Stools of a patient were studied daily for a period of several weeks to determine the type and quantity of eggs passed in the stools, before, during and immediately subsequent to a full course of antimony therapy. Preceding treatment the majority of the eggs were fully developed and viable, as determined by hatching tests, although a few immature viable ones and occasional calcified ones were also recovered. Rarely viable mature eggs were evacuated in pseudo-abscess and pseudo-tubercle adventitia. Treatment of the patient consisted of 75 cc. of fuadin, administered over a period of 16 days. Almost immediately after treatment was begun the number of eggs decreased but there was no change in the type of egg until the seventh day, when the number of viable eggs began to decrease rapidly and to degenerate, and dead or dying eggs, with larvae in various stages of development, became more and more conspicuous. By the twelfth day of treatment no living eggs were recovered but dead eggs in quantity were still being evacuated. On the fifteenth day there were no eggs recovered by concentration of 9 gms. of formed stool. On the nineteenth day the stool contained no dead or degenerate types but concentration of 8 gms. revealed a single, viable, immature egg, slightly less advanced than that illustrated in Figure 4. This study indicated the almost, but not quite complete success of the treatment, since at least one egg-laying female worm had survived the course of antimony. To the internist this indicated need for a second course of treatment. Thus the

prognostic significance of the laboratory findings is of particular importance as a guide to the efficiency of therapeutic procedure.

#### SUMMARY

1. This presentation acquaints the clinical microscopist with the relatively complex problem involved in the diagnosis of schistosomiasis japonica in so far as recognition of the eggs of the parasite are concerned.

2. Typical mature viable eggs of *Schistosoma japonicum* are not always found in the stools of patients infected with this blood fluke. In their place there may be immature eggs, either viable or degenerate, degenerate mature eggs or eggs in a pseudo-abscess or pseudo-tubercle capsule which has been enucleated from its location in the intestinal wall.

3. The process of egg laying in the mesenteric venules is described, together with the escape of the eggs from the venules, their filtration through the submucous and mucous coats of the intestinal wall and their appearance in flecks and strands of mucus and in the feces of the evacuated stool.

4. Detailed descriptions are provided of the immature and mature viable eggs, immature and mature degenerate eggs, nests of eggs trapped in mucus, eggs recovered from intestinal nodules and from dilated intestinal capillaries, eggshells, and the type of spine or protuberance which at times characterizes the shell of the egg of *S. japonicum*. These stages and types are illustrated by camera lucida drawings and by a photomicrograph.

5. The diagnostic and prognostic significance of the relative number and types of *S. japonicum* eggs recovered from the stools of patients undergoing treatment is indicated by a representative case in which careful quantitative and qualitative study of the eggs was made before, during and immediately following a 75 cc. course of treatment with fuadin.

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## AN ATTEMPT BY FEEDING TO INDUCE IN ANIMALS REACTIVITY TO TRICHINELLA SPIRALIS IN THE ABSENCE OF INFECTION<sup>1</sup>

JOHN W. AVERA, M.D., ELLARD M. YOW, M.D., GEORGE T. HARRELL, M.D., AND ELIZABETH B. FOWLER

In a previous study it was discovered that a significantly higher incidence of positive trichinella skin tests existed among patients confined in tuberculosis sanatoria and hospitals for mental disease than among patients in general hospitals (1). The length of stay in an institution seemed to be significant, since the incidence of positive reactions reached a peak in patients who had been confined for eighteen to twenty-nine months. Institutional cuisine is notoriously poor, and trichinosis may be transmitted by the inadequate cooking of large portions of meat, which leaves the center infectious even though the surface appears thoroughly done. The possibility of unrecognized subclinical institutional epidemics from this cause was considered, but no confirmatory evidence was uncovered.

It has been shown that the antigen contained in pollens can cross the mucosal barrier of the intestinal tract after ingestion, and produce symptoms in allergic individuals (2). If the sensitizing antigen can pass the intestinal mucosal barrier, the ingestion of meat containing killed trichinae might lead to the development of skin sensitivity. Cooking adequate to render trichinae non-infectious might denature the protein of the parasite, but meat rendered non-infectious by freezing could be so incompletely cooked as to leave the antigen unaltered. This mechanism might be the explanation for the high incidence of positive trichinella skin tests among institutionalized patients.

The present experiments were undertaken to determine whether positive trichinella skin tests could be produced in animals in the absence of infection by the ingestion of killed trichinae.

### MATERIALS

#### *Animals*

Forty guinea pigs weighing 250 to 350 gms. were fed a stock diet (Rockland guinea pig diet, Vitamin C fortified. Arcady Farms Milling Co., Chicago, Illinois) with a supplement of green vegetables three times weekly. The animals were kept in individual cages and were maintained on the diet for two weeks before the experiment was begun.

Thirty-eight rabbits, varying in weight from 2½ to 6 pounds, were fed a stock diet (Kasco complete rabbit ration, Kasco Food Co., Cincinnati, Ohio) supplemented with green vegetables daily. The rabbits were divided into five groups as shown in table 1; each group was kept in a separate cage.

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Albino rats weighing about 100 gm. were fed a stock diet (Rockland rat diet, Arcady Farms Milling Co., Chicago, Illinois). The groups of infected and non-infected animals were kept in separate cages.

### Meat

The strain of *Trichinella spiralis* was obtained from the National Institute of Health and was carried in rats. The density of infection in the diaphragm was maintained fairly constantly at 10 to 20 larvae per microscopic field.<sup>2</sup>

TABLE 1

GROUP	NUM- BER OF ANIMALS	MEAT	NUM- BER OF FEED- INGS	POSI- TIVE SKIN TESTS	POSITIVE SERUM FLOCCULATION TESTS
Guinea pigs					
A <sub>1</sub> Test group.....	6	Infected, frozen	16	0	0
A <sub>2</sub> Test group.....	5	Infected, frozen	1	0	0
B <sub>1</sub> Test group.....	6	Infected, cooked	16	0	0
B <sub>2</sub> Test group.....	5	Infected, cooked	1	0	0
C <sub>1</sub> Negative control.....	3	Non-infected, frozen	16	0	0
C <sub>2</sub> Negative control.....	3	Non-infected, frozen	1	0	0
D <sub>1</sub> Negative control.....	3	Non-infected, cooked	16	0	0
D <sub>2</sub> Negative control.....	3	Non-infected, cooked	1	0	0
E Positive control.....	6	Infected, untreated	2	5*	6 doubtful
Rabbits					
F Test group.....	11	Infected, frozen	39	1*	1*
G Test group.....	11	Infected, cooked	39	0	0
H Negative control.....	11	Non-infected, frozen	39	0	0
I Negative control.....	3	Non-infected, cooked	39	0	0
J Positive control.....	2	Infected, untreated	3	2*	2*

\* These animals were subsequently proven to be infected by muscle press or digestion technique.

Non-infected rats were starved for two days and then fed 1 gm. of infected rat diaphragm. The animals were not fed again until the infected meat had been consumed; the stock diet was then resumed. After twenty-one to twenty-eight days, the animals were killed by a blow on the head, skinned, and eviscerated. The muscle was stripped from the bone with scissors, and minced; that fed the the guinea pigs was placed in a food chopper and ground finely. Meat prepared in this manner was fed to groups E and J (table 1) without further treatment, for use as a positive control.

For test groups, A, B, F, and G the ground or minced meat was divided into

<sup>2</sup> Muscle press preparations were examined with 12.5× ocular and 10× objective lenses.

two portions. One was placed in a covered petri dish with two to three table-spoonfuls of tap water, and left in an electric hot air sterilizer for one hour at 60°C. (3). The second portion of meat was placed in a petri dish and packed in dry ice at -78°C. (4) for twenty-four hours. Prepared meats were never kept longer than one week and were stored in an electric refrigerator at 10°C.

Non-infected meat to be used as a negative control in groups C, D, H, and I was prepared in the same manner from non-infected rats. The diaphragm and portions of several skeletal muscles were first examined in the muscle press to be sure that no trichinae were present.

### *Antigen*

Part of the diluted antigen used in this study was supplied by Lederle Laboratories, Pearl River, New York, and by the National Institute of Health, Bethesda, Maryland. Part of the dried larvae used was furnished by Parke, Davis and Co., Detroit, Michigan. The remainder of each was prepared in our own laboratory.

Rabbits were fed trichinae contained in infected rat meat. After eight weeks the animals were bled from the ear vein and the serum was separated for use as a positive control in the flocculation test. The animal was then killed, skinned, and eviscerated; the muscle was removed and digested at 37°C. for six hours in a solution of 0.7 per cent pepsin and 1 per cent hydrochloric acid (5). The larvae were washed repeatedly in normal saline until they were biuret-negative; they were then dried in a desiccator and powdered.

For the flocculation test the powdered trichinae were used without further treatment. For skin testing a 1:200 weight/volume emulsion was made with the dried powdered larvae, and was allowed to stand for one week at room temperature. The supernatant fluid was then removed, placed in sterile vials, heated for one hour daily on three successive days in a water bath at 60°C., and tested for sterility;<sup>3</sup> 0.04 per cent phenol was added as a preservative.

## METHODS

### *Feeding*

Meat was placed in the mouth of the animal with forceps and the animal was held until the meat was chewed and swallowed. The guinea pigs were fed  $\frac{1}{2}$  to 1 gm. of meat on each occasion. Since it is known that trichinosis can be induced by a single feeding, part of the guinea pigs were fed killed trichinae only once (groups A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub>, D<sub>2</sub>). The positive control group (E) was fed twice, once at the beginning of the experiment and again six weeks before the flocculation test was performed. All other guinea pigs (groups A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub>, D<sub>1</sub>) were fed twice weekly for thirteen feedings and then were given three additional feedings over a period of three months.

Each of the rabbits in four groups (F, G, H, I) received approximately 1 gm. of meat three times weekly for a period of thirteen weeks. The larger number of

<sup>3</sup> Part of the diluted antigen furnished us was sterilized by filtration through a six pound Mandler filter.

feedings was used because the smaller number tried in the guinea pigs had been found to be ineffective. The known positive control group of rabbits (group J) was fed only three times.

### *Skin tests*

In order to avoid the possibility of sensitization, no animals were skin-tested before the experiment was begun. Two weeks after the conclusion of the feedings the flanks of the animals were clipped and the injections were made the following day. Each guinea pig was tested with 1:10,000 and 1:200 trichinella antigen (Lederle). Each rabbit was tested with trichinella antigen in dilutions of 1:10,000 (Lederle), 1:8,000 (N. I. H.), and 1:200 (Parke, Davis, Lederle, and our own). Normal saline was used as a control. The reactions were read at twenty minutes and twenty-four and forty-eight hours. All antigens used were found to be active and gave positive skin reactions in an infected dog.

### *Flocculation tests*

Two weeks after the skin tests were done, the guinea pigs were anesthetized with ether and 5 cc. of blood was obtained by cardiac puncture. Only 2 cc. of blood was withdrawn from the rabbits—in some cases from the ear vein, in others from the heart. The serum was separated and inactivated by being kept at 56°C. for thirty minutes. The tests were performed by the technique described by Suessenguth and Kline. (6). As a control, tests were done on known positive and known negative sera obtained from guinea pigs and rabbits. A sample of hemolyzed blood obtained from a non-infected rabbit was also tested in order to determine whether false positive reactions could occur as a result of hemolysis; this gave a negative reaction.

## RESULTS

### *Guinea pigs*

The animals in groups A, B, C, and D gained weight to an average of 758 gm. Two animals fed frozen infected meat (group A) died of intercurrent disease; the remainder appeared healthy for the duration of the experiment. Skin tests done two weeks after the completion of feedings were uniformly negative at twenty minutes and twenty-four and forty-eight hours. Flocculation tests on blood serum drawn one month after the completion of feedings were all negative. No trichinae were found in any animal by the muscle press or digestion techniques when they were sacrificed at the conclusion of the experiment.

Four of the six guinea pigs in the positive control group (E) gave 1 plus positive skin tests to 1:200 antigen at twenty minutes, and one gave a 2 plus reaction. The tests were negative at twenty-four and forty-eight hours. Six weeks after a second feeding of known infected meat, two animals gave 1 plus reactions and one gave a 2 plus reaction at twenty minutes; four gave 1 plus reactions and one a 2 plus reaction at twenty-four hours; but all were negative at forty-eight hours. All six guinea pigs in group E gave doubtful flocculation tests six weeks after the second feeding; known positive and known negative control sera from

rabbits and known negative guinea pig serum gave clear-cut positive and negative reactions.

### *Rabbits*

With the exception of the two rabbits in the positive control group (J) and one rabbit in the group fed frozen infected meat (F), the animals gained weight. All of the skin tests were negative at twenty minutes and at forty-eight hours. At twenty-four hours, the two rabbits with known infections [(group J)] gave positive reactions to the various antigens. The reactions ranged from pale 9 mm. wheals (1 plus) to 1 cm. wheals with induration and erythema (3 plus). The other rabbit which had not gained weight gave a 1 plus reaction to two of the 1:200 antigens.

Flocculation tests on undiluted serum from the three rabbits which gave positive skin reactions were positive (4 plus). Serum from all other rabbits in the experiment, and control serum from normal rabbits, gave negative flocculation tests.

The animal from group F which showed positive skin and flocculation tests died of pneumonia two weeks after the completion of the tests. Muscle press preparations of the diaphragm were negative, but digestion of 60 gm. of muscle—including the entire diaphragm and portions from the intercostal and pectoral muscles, and from all leg muscles—yielded one trichina. Undoubtedly, freezing did not kill all the larvae in at least one specimen of meat fed this rabbit. Only one other rabbit died during the course of the experiment; this animal was from the group fed cooked infected meat (G), but no trichinae were found by either the muscle press or the digestion technique. Additional rabbits from all groups were killed after the completion of the experiment, but no trichinae were demonstrated by the muscle press or digestion technique except in the control animals known to be infected.

### DISCUSSION

Reaction to an antigen which had crossed the mucous membrane of the intestinal tract would explain the high incidence of positive skin tests to trichinella antigen previously found in institutionalized patients. Spindler and Cross reported that skin sensitivity could be acquired by hogs from the consumption of scraps of pork containing non-viable trichinae (7). Hogs fed cooked trichinous meat over a period of several weeks gave weakly positive intracutaneous reactions to trichinella antigen. The animals were slaughtered at the end of the experiment and examined for trichinae; none was found to be infected (8). We have been unable to reproduce this phenomenon in guinea pigs or rabbits. Skin tests with several antigens, as well as flocculation tests on serum, failed to demonstrate evidence of reactivity in animals fed cooked trichinous meat. Since, theoretically, cooking might denature the antigen and render it non-reactive, animals were also fed trichinae killed by freezing—a process which theoretically should not denature the protein. Skin tests with several antigens, as well as flocculation tests on serum, failed to demonstrate evidence of reactivity.

Ingested pollen has been shown to be inactivated by gastric digestion in human beings (2). We have not investigated the ability of the digestive juices of herbivorous animals or of human gastric juice to inactivate the antigens of killed trichinae.

Since the degree of allergy in trichinous individuals is high, the passage of undenatured antigen across the mucous membrane might cause vague symptoms in such persons. These symptoms might be attributed to an allergy to pork, when actually the allergy is to the parasite contained in the meat.

It is conceivable that the mechanism tested by these experiments is the explanation for the high incidence of Brucella skin tests in relation to the reported incidence of brucellosis as proven by culture. Antigen which has been rendered non-infectious by pasteurization, if it passed the mucosal barrier, might still induce sensitivity without infection.

Contrary to the reports in the literature, we have been unable to produce strongly positive skin reactions to 1:200 trichinella antigen in guinea pigs known to be infected with trichinae (9). We have no explanation as to why the flocculation test was not clearly positive in infected guinea pigs. We did not do precipitin tests for comparison. The skin reactions were more satisfactory in the infected rabbits than in the guinea pigs, and the flocculation test was positive. The most clear-cut skin reactions were observed in the infected dog in which the potency of the antigens was tested. We would suggest that in future experiments concerned with skin reactivity rabbits, or preferably dogs, be used rather than guinea pigs.

The flocculation test proposed by Kline gave positive results in the rabbits with known infections in our experiment. This test detected infection of extremely mild degree in one rabbit from the group fed frozen infected meat. However, the technique is "tricky" and requires large amounts of dried, powdered antigen, which is time-consuming to prepare. In the present stage of its development, the test is probably not applicable to large-scale use.

#### SUMMARY

1. The repeated feeding to guinea pigs and rabbits of killed larvae of *Trichinella spiralis*, presumably containing denatured (cooked) or undenatured (frozen) antigen, failed to induce reactivity in the animals as measured by skin tests or flocculation tests of the serum.

2. Rabbits are superior to guinea pigs for experiments requiring skin-testing or flocculation of serum after infection with trichinae.

3. The flocculation test gives promise of being a useful test in suspected cases of trichinosis; an objection is that it requires large amounts of dried antigen.

4. This experiment did not afford an explanation for the unusually high incidence of positive skin reactions among patients confined in institutions.

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# DETECTING INTESTINAL PROTOZOA

## SALINE-IRON-HEMATOXYLIN SOLUTION FOR WET SMEARS

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It has always been a difficult undertaking to recognize and differentiate the cyst and trophozoite stages of the intestinal protozoa as they exist in human feces. A slide preparation consisting of a small portion of feces emulsified in a drop of physiological saline proves to be a mystery to the inexperienced worker; the separation of the true protozoan cysts or trophozoites of the amoebae and flagellates from the pseudoparasites and normal fecal debris is extremely difficult. Keeping in mind the complexity of detecting protozoa in stool specimens, it appears that a method of demonstrating parasites by contrast staining and complete separation from fecal material would definitely aid both the inexperienced student and the parasitologist. Attempts have been made along this line; neutral red or eosin creates a contrast between stained fecal material and the cysts or trophozoites of the intestinal protozoa. However, contrast has been incomplete and the parasites remain mixed with the fecal particles.

### SALINE-IRON-HEMATOXYLIN

The trophozoites of all intestinal protozoa will disintegrate in a very few moments if they are subjected to the osmosis of a hypertonic solution; therefore, the primary objective must be to use a solution that is isotonic. A second objective centers around the necessity of separating the cysts and trophozoites so they will remain free of coagulated debris. The final objective is to create a contrast between shiny, refractile cysts and trophozoites and a stained background, with the parasites remaining in a clear field. Saline-iron-hematoxylin satisfies these objectives; the solution is isotonic; the protozoa are separated from coagulated fecal debris; and the background is stained black, leaving the organisms their characteristic refractility.

### TECHNIC

To 75 ml. normal saline solution, add 10 to 15 ml. of 0.5 per cent hematoxylin stain solution (made according to the technic for iron hematoxylin stained permanent mounts) and 0.25 ml. (about six drops) of 4 per cent ferric ammonium sulphate; mix and this solution is ready to use. Place a drop of the saline-iron-hematoxylin on a glass slide. Select a small particle of feces with a tooth-pick or applicator and stir it in the drop of solution until a smooth emulsion is formed. Place a cover slip over the preparation, avoiding air bubbles. The film should be thin and uniform so that, when seen under the microscope, the Protozoa appear

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separate from the stained background and fecal particles. The solution should be shaken each time before it is used to prepare another smear. A blue glass filter should be used with the source of light to increase the effectiveness of this method. The solution remains satisfactory for an indefinite period, but it is best to make up a fresh solution every week or two since there is always the possibility of contamination.

#### SUMMARY

Saline-iron-hematoxylin has proved very satisfactory in teaching large numbers of Navy laboratory technicians the knack of identifying intestinal protozoa; less time is required than when the normal saline direct smear is used. From repeated observations, it is the general opinion that there is less eye strain, especially where several hours must be spent each day in examination of specimens. This solution has proved valuable in survey work. A positive specimen is detected more quickly, and the characters of the protozoa appear to be emphasized without any effect on trophozoite activity.

# A UNITARIAN VIEW OF TREPONEMATOSIS<sup>1</sup>

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It is the purpose of this sketch to present the human disease treponematosiis as it might be given in a brief textbook of medicine<sup>3</sup>.

## DEFINITION

Treponematosiis is a universally distributed acute and chronic specific infectious disease, known in various times and places by many names, such as syphilis, yaws, pinta, bubas, button scurvy, morbus gallicus, bejel, morfea, pian, irkintja, franghi, mentagra, carate, frambesia, venereal leprosy, sibiens, empeines and radesyge. Caused by a treponeme and propagated both venereally and non-venereally, it is susceptible to treatment with the heavy metals, is diagnosed by special tests, is characterized by an early and late stage separated by a latent period, and evokes a characteristic pathological response from human tissues.

## HISTORY AND GEOGRAPHY

Treponematosiis had its origin somewhere in the remote and unknown past. The biological relatives of the treponeme are saprophytes, and it probably established itself first as a parasite of man in some moist hot climate such as Central Africa, by entrance through a break in the skin. Since the body's reaction to the invasion is to break out with sores in which treponemata teem, subsequent propagation by contact, mostly among children, was easily accomplished under the conditions of human life which there prevailed. The treponeme thus became an obligate parasite of man and thrived in an environment which, failing to provide isolation or treatment, favored transmission and propagation.

As the disease in the course of thousands of years was carried by migration, commerce and the slave trade to other continents and more temperate climates, the improvement in sanitary conditions, especially in the temperate zone and in urban and civilized communities, gradually restricted the treponeme to transmission for the most part from one adult to another through sexual intercourse.

In the ancient and medieval world infectious diseases were grouped under the headings of sores and fevers. Much of the treponematous infection was obscured among the childhood exanthemata, though there are occasional references to disease conditions related to sex. With the discovery of printing, the upsurge of scientific thought and the quickening of men's minds by the explorations in

<sup>1</sup> Read before the thirty-eighth annual meeting of the American Society of Tropical Medicine, November 10, 1942 at Richmond, Va.

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<sup>3</sup> A full presentation of the subject will appear in a forthcoming chapter of *Oxford Medicine*.

Africa and the New World in the latter half of the 15th Century, the inchoate mass of infectious disease began to break up into its constituent elements, the small pocks among others, and the great pocks known as morbus gallicus.

With pardonable rationalization the groping science of medicine, emerging from the background of herbalist, alchemist and barber, linked the emergence of this disease with the discovery of the New World, though Fracastoro, the father of epidemiology, who himself introduced the word syphilis, stated that the simultaneous appearance of morbus gallicus in so many European countries ruled out the probability of its importation by Columbus and his men.

Ruiz de Isla, who was one of the first to write about the newly recognized disease, described the early and late stages accurately but introduced a third stage characterized by high fever and heavy mortality, obviously some intercurrent contagion such as typhus. In the course of a few decades the clinical picture of the two-stage treponematosi s became clearer and the naive conclusion was reached that the disease had become milder. What had really happened was that its diagnosis had become more accurate.

Treponematosi s in all probability was present also in the New World before the recording of history began. Columbus was by no means the first human contact between the Eastern and Western Hemispheres. Be that as it may, the slave trade brought a huge mass of treponematous infection to the New World, part of which still persists in nonvenereal form in certain rural areas of the Caribbean and elsewhere, and part of which has evolved into the venereal form in the negro of the southern States.

In the course of the centuries that followed the discovery of America, great progress was made in the study and treatment of venereal treponematosi s, which was known as syphilis. However, medical authorities became preoccupied with this form of the disease to such a degree that they came to think of syphilis as the whole disease and began to give other names to the nonvenereal type. This was how the native African word yaws came into medical usage.

In those days, before the discovery of the blood tests and the treponeme, when differentiation of diseases had to be made on their clinical appearance, this was an understandable position, because in some respects yaws certainly looked different from syphilis. Another error into which the scientists of that day fell was to elevate an unusual symptom into a disease, as was done, for example, with gangosa until it was finally recognized as a late sign of treponematosi s. Much the same thing is being done with pinta today, viz., erecting one feature of a protean disease into a disease *sui generis*.

When outbreaks of nonvenereal treponematosi s occurred in Europe, as occasionally happened under conditions of extreme squalor and destitution, they were called "syphiloids" and their nonvenereal character was recognized, but as conditions in each case improved, the nonvenereal type faded out, evolving into the venereal type.

Since the turn of the century much attention has been devoted to venereal treponematosi s (syphilis), because of absorbing questions of diagnosis and treatment and because of emphasis on its social and moral aspects. Less attention

has been paid to nonvenereal treponematosi (yaws *et al*) because, in contrast, it does not present problems of diagnosis, treatment and morals. This dichotomy has caused a concentration on venereal disease and elevated an epidemiological feature (venery) into a criterion of scientific classification. Specialists in this field actually came to be called venereologists.

However, this unbalanced emphasis is becoming less convenient, especially in these days when the world is shrinking so rapidly, and when U. S. medical officers are seeing nonvenereal treponematosi in so many tropical countries in this hemisphere, in Africa and in the Near and Far East. Manson's Tropical Diseases which represents conservative consensus states (1940) that the organisms of syphilis and yaws are indistinguishable, and the lesions produced by them extremely difficult to differentiate. Yaws, it says, "is now thought to be merely a primitive and tropical form" of syphilis. It is becoming generally more common to hear and see references to the "common ancestry" of the pathogenic treponemata. This is a welcome development, for it is certain that scientific knowledge will be gained and human welfare advanced by emphasizing their common properties rather than their alleged differences.

#### ETIOLOGY

*Treponema pallidum* (Schaudinn, 1905), syn. *T. pertenue* (Castellani, 1905), syn. *T. carateum* (Brumpt, 1939), etc., is a translucent and fragile spiral organism, which has either never been cultured or else reverts in culture immediately to a saprophytic, nonpathogenic form. Though the morphology and motility of *T. pallidum* are characteristic and remain remarkably constant, its physiological, functional and biological characteristics on the other hand are notoriously multiform.

*Treponema pallidum* was the name chosen by Schaudinn, but "spirocheta" became popular in spite of the fact that that word was preoccupied in zoological nomenclature by Ehrenberg in 1833. References to "spirocheta pallida" in scientific communications should be discontinued, since that term was a stillborn homonym and is therefore unsound and void. It would be useful in lay language to employ the words treponeme and spirochete interchangeably. It is common usage among clinicians today to call the parasite *Spirocheta pallida* when it causes the venereal form of treponematosi, but this does not affect the fact that *Treponema pallidum* is the correct scientific name.

It is obvious that such a versatile organism as *T. pallidum* is bound to produce strains or varieties under varying circumstances. Such strains are elicited by the epidemiological influences of venereal and nonvenereal treponematosi and run true to form so long as the respective conditions remain. The two main varieties of *T. pallidum* produce different clinical syndromes at given times and places, but their essential unity is demonstrated by the gradual evolution of one form into the other when environmental conditions change. These strains, in other words, are not static entities but the products of their environments, and variations within a species.

## EPIDEMIOLOGY

*T. pallidum* has no intermediate host and must make the transfer from one human body to another by direct contact of some sort. Further, since drying kills the parasite, the transfer must be made quickly and in a fluid medium. In tropic zones, among primitive rural peoples, ignorant of sanitation and hygiene, opportunities for direct contact of the smeared secretions of the body, especially of the skin eruptions, are innumerable, particularly among children who thereby become the reservoir of treponematous infection in those areas of the world. This accounts for childhood acquisition in yaws and bejel. On the other hand, in temperate climates and in urban and civilized communities the childhood reservoir dries up and treponematoses takes on a venereal epidemiology, the prostitute and a small segment of the adult male population becoming the chief reservoir. This is the background of occidental syphilis.

Whether venereal or nonvenereal treponematoses is predominant in a given locality at a given time depends on many environmental factors, climatic, economic, sociological and medical. There are innumerable variables in the three-sided equation of man, treponeme and environment. Both venereal and nonvenereal treponematoses may be present within a narrow compass, as for example syphilis in a town and yaws in the surrounding rural area.

Nonvenereal treponematoses may appear and disappear as local standards of hygiene fluctuate. In the course of time either type may evolve into the other, providing environmental conditions favor such a change. Sometimes this is a matter of a few years; sometimes several generations or several centuries may be required before the change is completed through innumerable steps of transition.

## SYMPTOMATOLOGY

Essentially, treponematoses presents symptoms which fall into two time zones, the early and the late. In many venereal infections, though not in all, the early stage may be further split into the initial lesion and the secondary rash. In either case the early stage consists of eruptions of skin and mucous membrane and the later stage is characterized by ulcers and specific granulomata of the bones, cardiovascular system, nervous system and the viscera. In general the nonvenereal type, as might be expected, produces overt lesions and symptoms, whereas the venereal form, partly because it is exposed to the hazards of treatment, tends to a more insidious course.

Congenital infection is commoner in the venereal type, probably because the mother usually acquires the disease within the childbearing period. In the nonvenereal type the fetus usually is spared by the passage of time intervening between childhood acquisition and later parturition.

## PATHOLOGY

In treponematoses recovery is balance, not cure; if a patient has the disease, he is immune, and if he is cured, he is susceptible. Again, the pathological manifestations of treponematoses often depend in a given case or area upon the manner

and the age at which it was acquired. In other words, even pathology follows on epidemiology. Thus there are differences in the histological picture in the early lesions of the two types of treponematosi, but analysis shows that there is fundamental agreement in the nature of the pathological response of human tissues to the presence of the treponeme. What the interaction of tissue and parasite finally produces in the way of pathological picture in each case is affected by immunological, physiological and anatomical factors, which vary with the environment, i. e., the epidemiology. So much depends on environment that there is less difference between syphilis and some forms of yaws than there is among the various forms of yaws itself. Syphilis has also shown great variation; the descriptions of the disease of fifty years ago fit present-day yaws better than they do modern occidental syphilis, and the syphilis of today throughout the world presents many different pathological aspects.

#### SUMMARY

The problem is largely one of interpretation and of agreement on nomenclature. The unitarian interpretation advanced herein suggests that arguments as to whether syphilis is yaws or not have no meaning when the paramount fact is grasped that both are forms of treponematosi. Diseases should be named if possible on the basis of their specific causes, which are assumed to be constant, and not on the basis of their epidemiological courses, which may fluctuate.

Once having established the disease treponematosi, caused by *T. pallidum*, it is reasonable and practical to divide it into its venereal and nonvenereal types if it is recognized that this classification is based upon environmental rather than etiological factors.

If syphilis is coming to mean venereal treponematosi, the same word can hardly continue to be applied to nonvenereal forms, such as yaws and pinta. The syphilologist will continue to treat venereal treponematosi called syphilis and caused by "spirocheta pallida", and the physician in the tropics will continue to treat the various nonvenereal infections by whatever name they are known locally, but the more doctors and pathologists think in terms of the whole disease and keep the key word *treponematosi* in mind, the sooner will confusion be cleared.





# THE INFLUENZA EPIDEMIC OF 1943-1944 IN SAN ANTONIO, TEXAS

MORRIS POLLARD, M. KALKSTEIN AND H. R. LIVESAY<sup>1</sup>

This is a brief report of the local manifestation of the nation-wide influenza outbreak of December-January 1943-1944 as observed in an army camp near San Antonio, Texas. The examination of serums was initiated just prior to the local outbreak and was continued for two and one-half months thereafter. Serum specimens from 175 cases were collected from all clinical cases of nasopharyngitis, atypical pneumonia, lobar pneumonia, and influenza, at the onset of illness and at weekly intervals for two weeks thereafter. These serum specimens were examined for the following diseases with the technics noted:

1. Influenza A and B by a modification of the Hirst test (1).
2. Cold Hemagglutination (2).
3. Ornithosis-lymphogranuloma venereum group<sup>2</sup> complement fixation test.
4. Q fever by the complement fixation test.

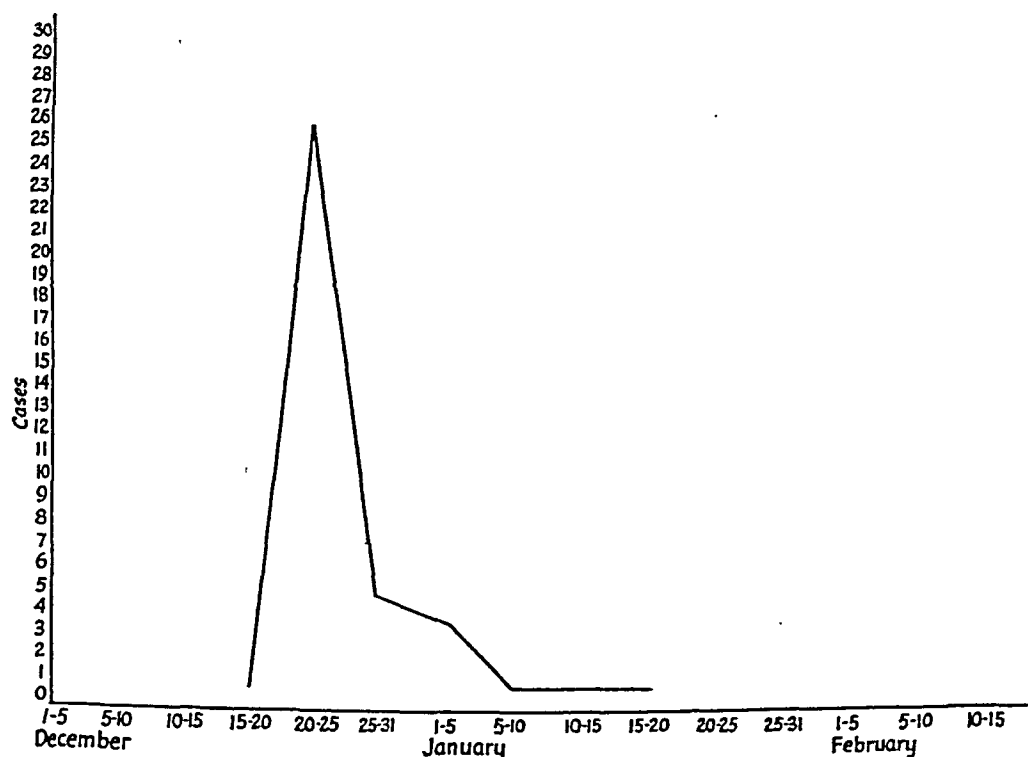


CHART I. INCIDENCE OF INFLUENZA A, MILITARY CAMP, SAN ANTONIO, TEXAS, DEC.-JAN. 1943-44

The findings in this brief survey were as follows:

1. This was an explosive outbreak of influenza A, one month in duration with 82 per cent of the 39 serologically positive cases occurring in the first two weeks. In this group are included eight which were diagnosed clinically as lobar pneu-

<sup>1</sup> Eighth Service Command Laboratory, Fort Sam Houston, Texas.

<sup>2</sup> Lygranum C. F. Squibb.

monia, thirteen as nasopharyngitis, and four as bronchitis. The average hospitalization period was 7.8 days with individuals ranging from two to fifteen days. The incidence curve as determined by the Hirst test showed a characteristic sharp "wax" and a gradual "wane" (Chart I).

2. Eleven of the influenza A cases (28.2%) showed x-ray evidence of pulmonary involvement ranging from "accentuated pulmonary markings" to "early pneumonitis of the lower halves of both lungs." Twenty-five cases were negative by x-ray examination.

3. The continued incidence of nasopharyngitis, "atypical pneumonia," and frank lobar pneumonia was unaffected by the abrupt cessation of influenza cases, as determined by serological procedures (Chart II). Twenty-four atypical pneumonia cases were diagnosed clinically as lobar pneumonia, three as nasopharyngitis, and one as bronchitis. The distribution of all diseases in the total examined serologically were 25% influenza, 33.6% lobar pneumonia, 45% nasopharyngitis, and 12.8% atypical pneumonia.

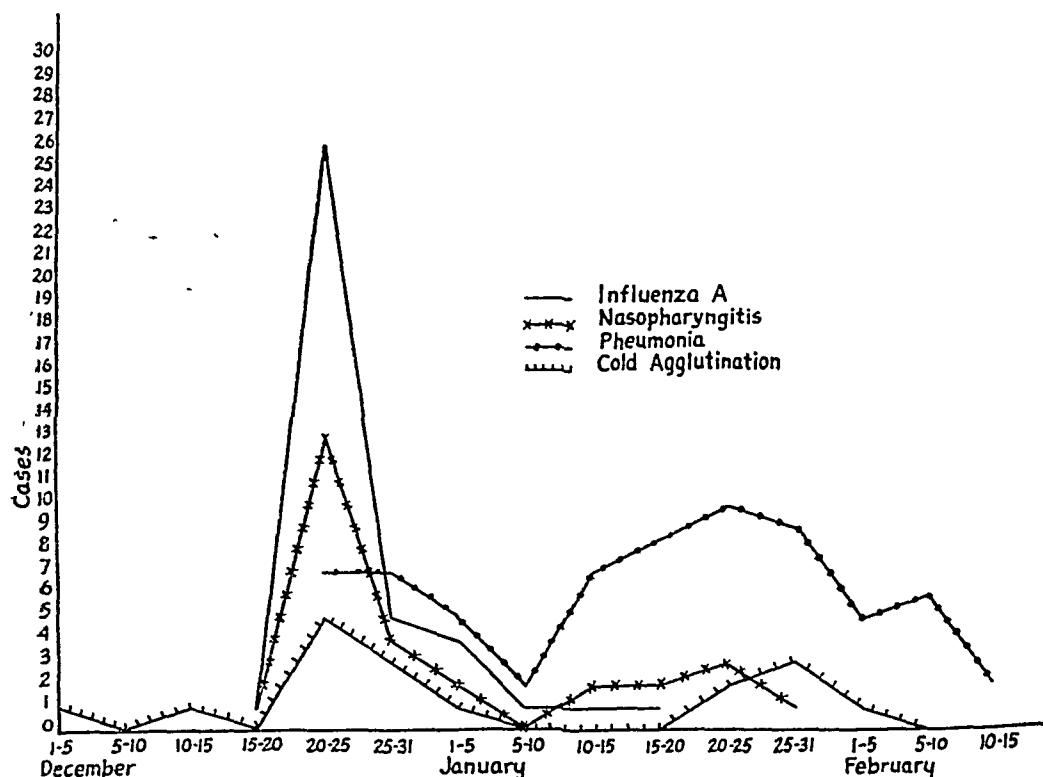


CHART II. UPPER RESPIRATORY DISEASES, MILITARY CAMP, SAN ANTONIO, TEXAS

4. No significant evidence of Lymphogranuloma-ornithosis infection or Q fever infection could be detected in this outbreak by the serological methods employed.

#### ACKNOWLEDGEMENTS

Grateful acknowledgement is made for the assistance and guidance in the selection of cases and collection of specimens to Lt. Col. W. T. Starr, M.C.

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# FOREIGN QUARANTINE IN MILITARY TRAFFIC<sup>1</sup>

PHILLIP T. KNIES<sup>2</sup>

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The dissemination of disease through international military traffic has been much discussed in recent years, and the problem has been evaluated from multiple viewpoints. Some persons have apparently been convinced that many diseases would be encountered abroad which might easily be introduced into the United States, particularly by aerial traffic, and fantastically devious means of infection have been suggested. Some opinions have not seemed to give adequate weight to past experience, or to improbabilities involved.

Military policy has necessarily been based upon careful evaluation of the entire problem by The Surgeon General with all reasonable regard to military expediency. World-wide commercial and military experience have been considered along with advice of American and foreign authorities in preventive medicine, and recommendations of the National Institute of Health, The National Research Council, the United States of America Typhus Commission, and other interested military and civilian groups. In an Interservice Committee for the Control of Exotic Diseases, the United States Public Health Service, and the Preventive Medicine Services of the Navy, and of the Army, have studied the subject repeatedly, and a detailed joint investigation of the matter was carried out in most of the accessible parts of the world through the Interdepartmental Quarantine Commission. This Commission was appointed by the Secretaries of War, and of the Navy, and the Administrator of the Federal Security Agency, upon the recommendations of the Surgeons General.

As a result, an extensive program of foreign quarantine has been undertaken by the Army, as a responsibility of The Surgeon General. A similar program has been initiated in the Navy, and continuing close correlation of military and civilian effort has been assured by the designation of Army, Navy, and Public Health Service Quarantine Liaison Officers. A Quarantine Branch has been established in the Preventive Medicine Service of The Surgeon General's Office and pertinent Army policy and directives will be published shortly. It is intended that uniform procedure extend throughout Army transportation, not only into the United States but also abroad, and it is believed the simplified techniques proposed will assure increased overall effectiveness. It is desired to present briefly the basic concepts and provisions of this program.

It may be emphasized that international quarantine, based upon centuries of experience and defined in the Pan-American and International Sanitary Codes, concerns only five diseases of proven virulence and epidemicity, namely cholera, smallpox, plague, typhus and yellow fever. Even though military traffic has

<sup>1</sup> Read at the Thirty-eighth Annual Meeting of the American Society of Tropical Medicine, St. Louis, Mo., November 13-16, 1944.

<sup>2</sup> Lieut. Colonel, M.C., A.U.S.

exceeded all precedent, reassurance with respect to these quarantinable diseases is provided by peacetime experience. Thus at the boundaries of the United States, its territories and possessions, the Public Health Service during ten years ending in April 1944 intercepted only nine cases of smallpox, two of epidemic typhus, and one each of bubonic plague and leprosy. Traffic during this period was reduced by economic depression, restricted immigration and war abroad, but only one hundred fifteen interceptions, more than half of which were of smallpox, had been made in the more normal decade of 1924 to 1934. Furthermore, in the history of aviation, there is record of the transportation across international boundaries of only one person ill with a quarantinable disease, a case of smallpox brought from South America to the United States last spring by commercial aircraft.

The emphasis placed by some upon highly improbable theoretical pathways of infection has neglected the basic principle upon which most epidemiological control is founded, namely the reduction of exposure factors below certain critical values. There is no reason to doubt the validity of this principle in the present context.

It may be further pointed out that most illnesses encountered abroad are the same as occur in this country, and that some of even the infectious diseases can be of little risk to the United States since there is already greater exposure, at least to endemic strains, than could conceivably be introduced in military traffic. For example, an average of more than twelve hundred cases of smallpox occur annually in this country, and of an American population of approximately twelve hundred lepers only about one-third are institutionalized. Our concepts of the prevalence of dread diseases abroad are often no less distorted than foreign opinion concerning our Rocky Mountain Spotted Fever, our field rodent plague in the West, and our encephalitis. Such distortion is based largely upon publicity accorded these diseases in medical literature. Actually, many military physicians who had hoped to instruct themselves in exotic diseases during foreign service have rarely encountered them except in native hospitals.

Nevertheless, there has been during this war an unprecedented potential exposure of American personnel to exotic diseases in places and in numbers unapproached before, except perhaps when large numbers of slaves were introduced at a time of sanitation less advanced than at present. The incidence among military personnel of some diseases, as leishmaniasis, onchocerciasis, schistosomiasis, and trypanosomiasis, has been negligible, but others have occurred importantly, as witness malaria, dengue, and in some areas scrub typhus and filariasis. Also, during this war there have been shortened times for international travel, unprecedented volume of traffic, and an urgency which might compromise sanitary safeguards during military operations. These factors must be evaluated in their proper stature.

Our defenses against exotic diseases are reassuring, for in addition to some inherent security, safeguards against infection from abroad were never more broadly nor more energetically erected. Danger from certain diseases is mitigated by the general sanitation and personal habits prevailing in this country,

and great improvement of the sanitary facilities of many communities has been achieved during the war through cooperation between public health agencies and the military forces. New high levels of protection against both water-borne and food-borne diseases have resulted. Climatic resistance naturally existing in the United States against many insect-borne diseases has been reinforced by systematic elimination of insect breeding by screening of houses of even the relatively poor and by extensive adult-insect control programs. These ideals have nowhere been more completely realized than in and near military communities.

Furthermore, military personnel are relatively segregated from the general public both during training in this country and upon return from abroad. While this segregation is partial only, its value is recognized in inquiries about protection of the public against diseases possibly carried by military personnel granted leave or furlough immediately upon return to the United States.

Other advantages of the military program of preventive medicine include immunizations against certain diseases and medicinal and mechanical prophylaxis against others. All persons in the Army are immunized against smallpox, typhoid and para-typhoid fevers and tetanus, and special immunizations are given against yellow fever, typhus, cholera, and plague in accordance with anticipated exposure. This policy offers obvious safety in the return of military personnel to this country.

A most important safeguard against introduction of disease by returning personnel is the continuous medical surveillance exercised over them. This consists not only in constantly available medical consultation, daily sick calls and monthly inspections, but particularly in special inspections upon change of stations, with appropriate corrective action when necessary in order to assure freedom from contagious disease and vermin. This barrier includes inspection before departure in international traffic whether by plane or vessel. At this time a certificate is required noting those diseases to which the individual or group can be presumed to have been exposed and assurance is given of freedom from contagious disease and from vermin of both the individual and his effects. Additional inspections are given on shipboard at stated intervals and before debarkation; equivalent inspections may be given in aerial traffic if circumstances require. The result is quarantine, not of the traditional terminal type but one beginning at the onset of travel and continuing until its conclusion.

Upon arrival in this country, there are additional examinations in accordance with the exposure indicated in the certificate made out upon departure from abroad, and in accordance with individual and group histories of illness. Because of the security afforded by immunization against the quarantinable diseases, these additional examinations need not be accomplished immediately upon arrival, in the manner of traditional quarantine, but may be postponed until more adequate time and facilities are available as at reception stations. Those individuals who depart on immediate leave or furlough and hence temporarily are not under military medical supervision, receive special examinations before such departure is permitted. They are also warned regarding diseases they might harbor and are instructed to report immediately to the nearest military medical officer or to

their own physician in case of illness. Persons in whom disease is discovered are hospitalized and adequately treated before furlough or separation from the service.

Other safeguards should be mentioned. While certain diseases do exist abroad against which we possess no inherent or prophylactic guarantees, the American soldier is protected by sanitation not only of the military bases but often of adjacent villages and cities as well, by his relative segregation in military bases, and by restriction of his visits to native quarters, particularly when diseases are epidemic or significantly endemic. Education of the soldier is directed at decreasing possible exposure, and of his medical officer at increasing early recognition. The interruption of unavoidable exposure at the earliest possible moment favors the development of mild infections with some diseases, such as filariasis and schistosomiasis, in this manner reducing danger to the individual and minimizing possibility of secondary spread.

Military personnel returning to this country normally depart from abroad and arrive in the United States at sanitized military bases. Conveyances are regularly cleansed, frequently inspected and, when necessary, are disinfected. Material likely to harbor vermin is disinfested chemically, by heat or by storage.

While the application of these protective mechanisms may at times be sub-optimal, it is believed that they have been adequate to the problem at hand. In the early phases of the war the protection of the large outgoing traffic of personnel could safely rely to a considerable degree upon the inherent health and sanitation of this country, while the relatively slight incoming traffic was handled without apparent untoward result to date.

In the meanwhile, defenses have continued to develop in the realistic employment of the best principles of preventive medicine and the public may be assured that while placing the highest value upon military action towards successful conclusion of the war, the military viewpoint contemplates no avoidable risk to sanitation or health.



## BOOK REVIEWS

*Clinical Parasitology*. CHARLES FRANKLIN CRAIG, M.D., M.A. (Hon.), Sc.D., F.A.C.S., F.A.C.P., Colonel, United States Army (Retired), D.S.M., Formerly Director, Army Medical School, and Assistant Commandant, Army Medical Center, Washington, D. C.; Emeritus Professor of Tropical Medicine in the Tulane University of Louisiana, New Orleans, Louisiana, and ERNEST CARROLL FAUST, M.A., Ph.D., Professor of Parasitology in the Department of Tropical Medicine, Tulane University of Louisiana, New Orleans, Louisiana; Consultant to the Secretary of War, Army Epidemiologic Board on Epidemic and Tropical Diseases; Consultant, U. S. Public Health Service; Honorary Consultant, Army Medical Library; Fourth Edition, Lea and Febiger, Philadelphia, Pa. 1945.

One reads successive editions of Craig and Faust's "Clinical Parasitology" with increasing respect for its authority. Unfailing in reliability, profound in scholarship, exact and painstaking in detail, the book is a monument to the scientific attainments of the authors and to their creative energy.

The new edition has been expanded by more than one hundred pages but the use of lighter paper has permitted this increase in material without increase in bulk. Twenty-one new figures have been added. As in the previous edition, the book is divided into six parts which, following a general introduction, deal in turn with protozoa and protozoan infections, helminths and helminthic infections, arthropods and human disease, technical procedures and references. A new chapter on the geographic distribution of parasitic infections appears in the general introduction. Much fresh material and new sections on pathogenesis are found in the accounts of the various parasitic and arthropod-transmitted diseases. The bibliography, always a distinguished feature of this work, has been amplified and brought up to date.

Not only in form but in content is the fourth edition worthy of its predecessors. The finest quality of the book continues to be its high degree of accuracy. Much of the material presented represents the original work of the authors on many parasitic species and diseases in many lands over many years. The scope and exactness of their observations is the essential basis of the book's authority.

The work is almost equally authoritative in its citations from the reports of other scientists, the completeness, fairness and precision of these citations giving the reader a comprehensive view of the entire field of human parasitology. It is inevitable that in these quotations a few errors should appear. One such is found on page 236 where it is implied that during the malaria epidemic in northeastern Brazil in 1938-1940, the infection rate of *An. gambiae* was 100%. Confirmation of this statement was not found in Soper and Wilson's monograph. These latter authors regarded sporozoite rates of 28.2% and oocyst rates of 71.5% reported by Souza Pinto as "extremely high".

The clinical accounts of parasitic diseases which comprise a large portion of the book are of a high order. One wishes that the authors would restrict the use of the word "parasite" to mean "animal parasite" and that there would be less reference to "toxemia" except in connection with the physiological effects of a specific demonstrable exotoxin of the type found in diphtheria, botulism, tetanus and Shiga dysentery, but these are matters of option.

The scientific authority of Craig and Faust's "Clinical Parasitology" is not always reflected in its style. "Hypothecated" (pp. 360, 361), "hemobilirubinemia" (p. 20) "most usually" (pp. 315, 754), the prepositional use of "nearby" (pp. 209, 543, 755) and phrases such as "the raw consumption of snails" (p. 448) are not the only lapses noted. Misspelled words are few except for "layed" which appears at least sixteen times, "laid" twice. Additional evidences of the difficulties of wartime bookmaking are found in "harmáttan" (p. 27), "parturate" (p. 362), "Kondolean" (p. 369), "coinspicious" (p. 552), "rivertine" (p. 680), "pruritus peronei" (p. 76S) and "Lactrodectus" (p. 770). Errors of reference occur on pages 447 and 703 and of typography on pages 74, 283, 520 and 769.

In view of the increasing appreciation by practicing physicians of the value of this text,

and their need for it in postwar medical practice, it might be worthwhile for the authors to consider making certain concessions in subsequent editions to the average physician's needs and to his limited knowledge of parasitology. The usefulness of the book as a work of ready reference could be increased by recasting the sections devoted to the rarer parasitic infections in small type. A similar makeup of the paragraphs on treatment could be adopted to give appropriate emphasis to preferred methods of therapy. Physicians would also appreciate the rigid use of USP, NF, NNR and BNA nomenclature, and the insertion of the maker's name when proprietary drugs are recommended. A short glossary of technical parasitologic terms unfamiliar to the average physician would also, it is believed, be helpful.

In closing, let it again be stated, that Craig and Faust's "Clinical Parasitology" is still unsurpassed in its field, and it can be confidently expected to survive many more reviews and reviewers.

ELLISTON FARRELL

# MEDICAL RESEARCH IN THE POSTWAR WORLD<sup>1</sup>

## PRESIDENTIAL ADDRESS

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There can be no question that World War II has given to scientific research an impetus such as has never before been felt in the search for new knowledge and in its application. I need not review for this audience the events which have engendered such a widespread concern for the advance of scientific investigation. Suffice it to say that the developments of the war years have brought to science a wide popular recognition and a public interest hitherto unknown in the United States.

The prosecution of the war required that the Federal Government concern itself extensively with research and development of technological application of new scientific discoveries. This has been true in past wars as far back as the Civil War. The vast scope of global conflict and the evolutionary advance of science, however, have combined to increase the Government's rôle in research during the past six years to a stage which overshadows all previous activities. One result of the new achievements in logistics has been to create in the minds of many people—including many scientists—the impression that Government's participation in scientific research is a war-born activity. The truth is, the Government is in research and development,—and has been since the early days of the nation. The expansion of Government research has been gradual but steady; it has reflected the evolutionary changes in civilization resulting from exploration of unknowns.

In medical research and related fields, the United States Government financed a great many long term programs prior to the war. Problems which have a bearing on human health have been investigated by several Government agencies. The contributions of these agencies to the study of tropical and other diseases are well known to this audience.

Organized medical research in the United States is a product of the twentieth century, although it had its roots in the efforts of those imbued with scientific curiosity during the latter part of the nineteenth century. American universities were among the first of our institutions to devote attention to medical research, along with Federal and State agencies. Later, private foundations—and still later, commercial laboratories—entered the field. Today, these organizations conduct practically all of the health and medical research in this country. The isolated scientist is now being placed at a disadvantage. This is largely, of course, because scientific progress has made it difficult to engage in research without facilities, equipment, and funds beyond the means of an individual to supply.

Financial support of research in medical fields has been derived chiefly from

<sup>1</sup>Read at Meeting in Cincinnati, Ohio, November 12-15, 1945.

three sources: private endowments, governmental appropriations (State and Federal), and commercial expenditures. The universities have financed the programs through a combination of sources—endowments, gifts, grants from public and private institutions, and (in the case of State universities) appropriations. In some instances, commercial firms have financed investigations in universities. Some of the private foundations not only make grants or gifts to other research institutions, but also conduct broad programs in their own facilities. Federal agencies engaged in research rely entirely upon appropriations, although some of them may accept gifts from outside sources or engage in cooperative studies. The Land Grant Colleges represent one of the earliest activities of the Federal Government in the support of State research agencies.

Comprehensive and accurate information as to the amounts spent annually upon medical research is extremely difficult to obtain. From partial data, however, certain trends prior to the war may be discerned. For example, between 1937 and 1940, grants by private foundations and individuals for research and training in medicine and public health decreased by well over one-fourth (27.3 per cent). In the latter year, these sources supplied about \$5,000,000 for these purposes. Governmental appropriations for medical research, on the other hand, have expanded during the past 10 years. For example, in the Public Health Service annual appropriations for research have increased from a few hundred thousand in 1935 to over \$3,000,000 in 1945; in addition, laboratory facilities have been constructed in the same period at a cost of some \$8,000,000.

During the war, many pressing problems made necessary an expansion of Federal support for research, as well as the establishment of a coordinating agency,—the Office of Scientific Research and Development. At the same time, the National Research Council—a World War I development—had to be activated with many special advisory committees. Medical research was an important element in the expanded wartime program. In the first three years of operation, the OSRD expended over \$15,000,000 in Federal funds for medical research, of which nearly half (seven and three-quarter millions) was allotted in the peak year, 1944, alone.

The results of the war research conducted under these programs have proved the value of intensive, coordinated investigation. The great lesson of this experience, with respect to postwar research, is that the program of the OSRD functioned smoothly because the universities, the Federal agencies, and other cooperating institutions—all were represented on the various advisory bodies.

The report of Dr. Vannevar Bush and his colleagues to President Roosevelt is well known to all of you. The National Advisory Health Council and the National Advisory Cancer Council in joint session adopted the following statement on the Bush Report: "It is a magnificent and distinguished document which outlines a plan for stimulating basic research in civilian research institutions and for continuing the close and profitable cooperation between civilian and governmental research agencies . . ." The aims and views expressed in the report were indorsed by the Councils.

As you know, several pieces of legislation have been introduced in the Congress to implement the objectives of the Bush Report.

Joint hearings have been held before Senate subcommittees on the Kilgore, Magnuson, and Fulbright bills. The first two—the Kilgore and Magnuson—would establish a National Foundation for the support of research; the Fulbright bill would complement the Foundation through the establishment of a Bureau in the Department of Commerce to promote and develop new industrial processes and products. The Kilgore and Magnuson bills are the two major proposals with respect to comprehensive research. Although there is substantial agreement as to the basic issues involved, there are certain areas of disagreement in these two bills,—namely, scope and purpose of the foundation; methods of control and administration; and patent rights in government-supported discoveries.

Along with other scientific bodies, the National Advisory Health Council and the Cancer Council have consistently held the view that a National Science Foundation should be established only if proper safeguards are maintained legally for the independent development of research programs in institutions receiving government grants.

If scientific research and development activities in public and private groups are to function harmoniously as a correlated program, active cooperation among all groups must be assured. We know that any research program can be coordinated by direction and control. But since “direction and control” may mean death to a research institution and to the work of individual scientists, such methods might terminate in the foundation’s having no research program to coordinate. Cooperation being the method of choice, it seems wise that a National Science Foundation should have the benefit of advice and consultation from the various groups concerned, including the Government’s research agencies.

In addition, research institutions, whether public or private, which receive support from the Foundation should be assured complete freedom of scientific thought and endeavor, independence to develop their own programs, and, finally, a minimum of paper work in connection with the maintenance of accounts and reports.

With adequate safeguards such as I have mentioned, it is hoped that increased support of medical research by the Federal Government will be provided as speedily as possible. To promote the training of scientists, the National Institute of Health has already established fellowships, some of which have been filled.

The necessity for expanded public support of research is clear. Funds for Federally financed projects now under way in universities cannot be cut off without causing a serious financial situation in these institutions. New research should be planned in order to assure continuity of accomplishment. It will serve to provide opportunities for the employment of the valuable scientific personnel who are now being released from war projects. The Nation can ill afford to throw aside the investment it has made in medical research during the war, with the concomitant loss of potential additions to medical knowledge. Finally, active and expanded programs of investigation in health and medical fields are essential if we are to discover and utilize fully the best young research brains in the country.

Many of the projects initiated during the war were undertaken because of the

need of solving problems which as yet have not been answered. These problems are of vital importance to the public health and much work upon them will be essential for some years to come. Among the investigations which must be pursued intensively are: studies in the tropical diseases, the chronic degenerative diseases, also cancer, gerontology, the virus diseases, and studies of antibiotics and chemotherapeutic agents.

Despite the great contributions of the past, our knowledge of many tropical diseases remains fragmentary. Especially needed are improved methods for the early diagnosis of such infections as filariasis, schistosomiasis, and the leishmaniasis. Treatment of these diseases also requires much intensive experimental and clinical research.

The continued reduction in infant mortality, as well as in death rates from specific causes such as pneumonia, syphilis, and other infections which respond to modern therapy, again points to the aging of the population and to the increased importance of heart diseases, cancer, tuberculosis, arthritis, cerebral hemorrhage and other diseases of middle and old age. The problem of aging is in urgent need of intensive study. Much must be learned regarding the processes which not only increase the death rates, but which lower the physical and mental capacities of the older members of the population.

The paucity of research in cardiovascular diseases has been emphasized on numerous occasions. Dr. Henry Simms of the College of Physicians and Surgeons told a Senate subcommittee in 1944 that less than 25 cents per death from heart disease is spent for research in that field, as compared with \$2.00 per cancer death, \$4.00 per death from all infectious diseases except poliomyelitis, and \$500 per death from the latter.

Research on cancer has been developed for a longer period and to a greater degree than has the study of any degenerative disease. In comparison with support of scientific investigations of other diseases, funds for cancer research, both from private and public sources, have been supplied generously. It would seem, from the results so far obtained, that science should now develop new approaches to the cancer problem. New men should be recruited and trained,—and young scientists should be encouraged to seek new methods and new approaches in the study of cancer. Among promising lines of investigations in this field, I would mention the search for a method for early diagnosis of the cancerous process. The National Advisory Cancer Council has emphasized this need in commenting upon the disastrous periods of delay between the first examination of a cancer patient by a general practitioner and referral to a cancer specialist; and, indeed, between the visit to the specialist and final application of therapy.

In the field of chemotherapy, basic research is needed to determine the mechanism of action of new specifics, such as the sulfonamides and antibiotics. The electron microscope and isotopes present means for the development of new methods and technics in this field. Intensive development of such new methods gives promise of the solution of many fundamental problems, such as virus diseases, the biochemical analysis of bacteria, and determination of the active ingredients in antibiotics.

It is safe to say that the next few years will see the development of many new antibiotics, the development and application of which will depend upon fundamental, long-term research. The discovery of penicillin may be called "a happy accident," but only by acquiring basic knowledge can this and analogous therapeutic agents be fully exploited for the benefit of the public health.

These are but a few of thousands of important, long-range projects in medical research which should be undertaken. Experimental and clinical studies by various groups should be initiated and conducted in the cooperative manner which has produced such remarkable results during the war.

The great progress which has been made and the larger promises of the future call for the continuing development in the post-war period of a national program for the support of scientific research and development, an important sector of which would be support of the health and medical sciences. The benefits of wartime collaboration by the government, the universities, foundations, and industry, must be extended to aid us in our great peace problems. Health and medical research in the war has brought science to new frontiers; it remains for all groups to join in pushing back these frontiers so that greater benefits of health may be enjoyed by all mankind.





TWO NEW SPECIES OF RAT MITES (NEOSCHONGASTIA SPP.)  
FROM A FOCUS OF SCRUB TYPHUS ON MINDORO,  
PHILIPPINE ISLANDS

CORNELIUS B. PHILIP<sup>1</sup> AND THEODORE E. WOODWARD<sup>2</sup>

*From the United States of America Typhus Commission, War Department, Washington, D. C.*

During the operations of the Visayan Task Force on Mindoro Island, the U. S. Army troops in the San Jose district experienced the largest number of cases of tsutsugamushi disease (scrub typhus) that was encountered in any locality in the Philippine Archipelago during the war. Incidental to a survey of contributory factors and other pertinent data in the San Jose area, the larvae of two new species of trombiculine, parasitic mites were discovered in the ears of indigenous field rats.

Both of these new species belong in the genus *Neoschongastia* (family Trombiculidae), the larvae of which have a pair of swollen sensillary appendages (pseudostigmatic organs) on the dorsal scutum, and are without dorsal serrations on the chelicerae.

It was not determined whether animals other than rats occur as normal or casual hosts of these mites on Mindoro island. In the cultivated areas about San Jose where most of the military installations were placed, nearly every individual examined of the predominant local rat, *Rattus mindanensis mindanensis*, carried larvae of one or both of these mite species in their ears. A few were found also on the smaller rat, *R. vigoratus*. However, one or the other mite usually predominated in a given local area without any distinguishable ecological differences. Attempted "boot collections" in these areas were without success.

Although these were the most abundant rat mites encountered, other species identified from rats included scattering *Trombicula deliensis*, and *Heaslippia* (*Trombiculoides*) *gateri* taken in one area by Captain Mangrum of the 38th Malaria Survey Detachment. The former was the probable vector of the infection in the troops.

Species of *Neoschongastia* are known to attack man in other localities, but no mites for identification were recovered from man in Mindoro during or after the local epidemic in troops described by us elsewhere (1946).

The two new species are here described for purposes of reference in discussion of scrub typhus in the Philippines in another report. Collection of specimens was aided by the officers and men of both the 31st and 38th Malaria Survey Units commanded by Captain Edward S. Ross, Sn.C., O-446989, C.O., 38 Malaria Survey Unit and Captain Wayne L. Howe, Sn.C., O-514094, C.O., 31st Malaria Survey Unit respectively. Identifications of rats were made through courtesy of Dr. Remington Kellogg, U. S. National Museum.

<sup>1</sup> Lieut. Colonel, Sn.C., AUS.

<sup>2</sup> Lieut. Colonel, M.C., ORS.

## NEOSCHONGASTIA PHILIPPENSIS N.SP.

A species whose larvae belong in the group with hairy, globose sensillae on the scutum, and distinguished by the very sinuous posterior margin of the latter, and the sensillae widely separated and each situated on broad, raised areas in undistorted mounts. In life, light yellowish red to pinkish yellow depending on state of engorgement.

*Holotype larva* ovoid, partly fed, length to base of palpi 232, width 88  $\mu$ . Scutal adornment typical of the genus, shape as figured. Standard data in  $\mu$  (capital letters correspond with table of variation of paratypes below). Scutal Depth (median saggittal) 30, Posterior Sensillary Base (to projection of line of most posterior scutal margins) 9, Anterior Width 50, Posterior Width (between bases of PL) 69, Sensillary Bases (between centers) 20, A-P (between bases lateral setae) 23, lengths of setae: Anterior Median 20, Anterior Lateral 41, Posterior Lateral 36, SENSillae 23, blade 16 by 16. Both sensillae are present so that in the mount the bases and surrounding scutum appears to have been somewhat depressed possibly contracting slightly the PW which is proportionately narrower than in the paratypes.

Dorsal setae, moderate in number, of subequal size and shape throughout, slender, tapering and finely ciliated: Dorsal Setae Anterior 28  $\mu$ , Dorsal Setae Posterior 28. Arranged in rows of 2, 6, 6, 6, 6, 2, 2. Capitulum, and its appendages normal for genus. Ventral setae sparse and small, posterior ones a little longer, the usual pair each between the first and third coxae, the caudal group not bilaterally symmetrical, 18 preanal (in apparent rows of 4, 6, 6, 2) and 13 from the anus backward. Anus ventral. Coxae unisetose. Setae of remainder of legs of 2 types, in addition to the usual rod-like setae dorso-apically on the first metatarsae, (a) the predominating slender setae, ciliate on the outer side only, and more or less appressed to the legs, and (b) a few dorsally situated, upstanding, straight setae (often broken), ciliate on all sides, the most prominent of which is one located dorso-basally on the fourth segment of the third leg (tibia III) and measuring over twice the length of the seta on the corresponding coxa. Tarsal claws normal, the middle digit more slender and a third longer than the two outer ones.

Taken from the ear of a rat, *Rattus mindanensis mindanensis*, captured in a focal area of scrub typhus, vicinity of San Jose, Mindoro, Philippine Islands, April 2, 1945. Deposited in the U. S. National Museum, Washington, D. C., Slide No. 1525. A slightly fed, smaller paratype under another, marked cover-slip on the same slide.

*Paratype larvae*. Characters as above. Most of these, depending somewhat on mounts, show a definite bulge in the scutum about the base of each sensilla so that the area between them, as well as the prolongations around the bases of the lateral setae are depressed. There thus often appears to be a median, longitudinal furrow in the scutum. Fed specimens measure up to 292 by 400  $\mu$  (from caudal margin to base of palpi), relatively unfed specimens 120 by 120  $\mu$ . Standard data of 10 specimens show the following variation compared to the type.

SD*	PSB	AW	PW	SB	A-P	AM	AL	PL	SENS	DSA	DSP
32	7	49	72	22	22	21	40	40	26	28	28
30	8	48	72	20	20	24	32	48	25	26	26
32	9	51	76	24	22	28	44	48	24	30	30
33	12	55	80	27	24	28	48	44	30	31	30
26	10	51	74	22	22	28	45	46	28	29	28
	9	54	79	23	21	27	41	42	28	28	26
26	8	51	74	24	21	23		42	26	28	28
32	9	48	70	22	22			44	25	26	28
30	9	48	73	21	20	28	38	44	26	28	26
32	9	54	76	25	22	25	42	47	30	31	29

\* Meanings of abbreviations in headings are explained in text.

Measurements are affected by frequent tilting of the scuta in fed specimens, by setae set at angles or curved, so that minima in the above table are undoubtedly well under the actual figure. The maximum of the sensillae represents total length when the stalk is extended, though in usual mounts of this species the stalks are contracted and sinuous.

Paratypes include one larva on the holotype slide, plus 6 other slides with 86 specimens from rat's ears from the type location. Three slides with 69 specimens from ears of rats captured April 3, 1945, in another locality of the San Jose area, Mindoro, P. I. In the collections of the U. S. National Museum, South Australia Museum, Philippine Bureau of Science (if and when reestablished), the Rocky Mountain Laboratory of the USPHS, and the authors.

*Hosts.* *Rattus mindanensis mindanensis*, and a few off the smaller *R. vigoratus*. No records of casual parasitism of man.

#### NEOSCHONGASTIA KOHLI N.SP.

This species is also related to the group with hairy, globose sensillae on the scutum. The sensillary pits separated by less than their diameters, plus the anterior lateral setae longer than the posteriors, separate this from related forms. Color in life as in *N. philippensis*, and often associated with that species in the ears of rats in Mindoro.

*Holotype larva*, ovoid, slightly fed, length from base of palpi, 162, width, 131  $\mu$ . Capitulum and its appendages characteristic of the genus, the chelicerae with a minute subapical tooth on each. Setae on palpi all ciliated. Scutum as figured, posterior margin flattened mesally. Standard data in  $\mu$ : SD 35, PSB 7, AW 50, PW 62, SB 9, A-P 19, AM 22, AL 44, PL 33, SENS 20 by 16. Sensillary bases in bits depressed below scutal level, the short stalks of the sensillae not included in the preceding measurements therefore.

Dorsal setae stout, numerous, strongly ciliated and increasing in size caudally, DSA 27  $\mu$ , DSP 42. DS count 10, 4, 13, 11, 12, 10, 8, (9). The 2 anterior rows are peculiar in that the customary lateral pair are replaced by 2 pairs situated behind rather than in front of the first continuous row. The last group of 9 setae is not arranged in obvious rows owing to lack of abdominal distention. Anus ven-

tral in the caudal third. Ventral setae in preanal median area small, tapering, ciliated, markedly increasing in size caudally, rows irregular, the usual pairs between the first and third coxae plus 40 in the preanal area, and about 14 from the anus to the posterior margin. The exact count of the posterior ventral setae is difficult because of the compactness and density of the overlying dorsal setae.

*Coxae unisetose.* Legs with normal vestiture, the setae all normally ciliate on the outer sides only. Tarsal claws normal as in the preceding species.

Taken from the ear of a rat, *Rattus mindanensis mindanensis* captured in another than, though similar to the focal area of scrub typhus, where the type of *N. philippensis* was taken, vicinity of San Jose, Mindoro, Philippine Islands, April 2, 1945. Deposited in the U. S. National Museum, Slide No. 1526, the type marked in the glass. On the same slide are 9 paratypes plus one larva of *N. philippensis*.

*Paratype larvae.* Characters as in the type. Engorged specimens measure up to 360 by 448  $\mu$ . The dorsal rows of setae are quite prominent in partly fed specimens, but both the count and their regularity vary. Occasional adventitious setae are to be seen, often out of line and confusing in slightly fed specimens especially caudally. In most specimens, the 2 pairs of sublateral setae are well separated behind and beyond the first row. Variation in counts of respective dorsal rows of 16 specimens in all stages of engorgement are: Row I, 8-11; III, 9-12, usually 10; IV 9-12, usually 10; V, 10-13, usually 12; VI, 8-10, usually 8, VII and VIII when separable in at least partially fed specimens 4 to 6, and 4. Ventral setae consist of about 30 to 35 preanals and about 16 peri- and postanals. Anus is never apical.

Standard data of 11 paratypes show following variation.

SD	PSB	AW	PW	SB	A-P	AM	AL	PL	SENS	DSA	DSP
32	8	51	64	10	19	20	48	32	20	28	46
32	8	50	60	9	20	25	44	35	24	28	44
33	9	54	64	10	18	20	42	36	24	30	44
32	9	52	64	10	18	24	46	32	22	30	42
34	9	56	66	9	21	24	47	40		30	46
35	8	53	64	10	21	27	48	36	22	30	44
32	8	48	60	9	21	20	46	32	20	29	42
32	8	54	64	9	19	20	42	33	28	28	41
35	10	53	65	11	20	22	48	34	29	28	40
32	10	52	64	11	20	20	44	32	27	26	?
32	7	52	65	8	20	28	45	35	20	28	42

As in the preceding, minimal measurements especially of setae may be due to tilting or curvature. The sensillae measure above 24 only when stalks are extended or they are lying loose from their bases.

Paratypes all taken from the ears of rats near San Jose, Mindoro, both in focal and non-focal areas. In addition to 9 paratypes on the type slide, there are 112 paratypes on 10 slides from same locality, and host distributed among collections of U. S. National Museum, South Australia Museum, Philippine Bureau of

Science (when and if reestablished), Rocky Mountain Laboratory of USPHS, and author.

*Hosts.* Same as for the preceding species. The species is named for Major Glen M. Kohls, Sn.C., A.U.S., a colleague in the U. S. A. Typhus Commission who has contributed much fundamental information to acarology.

#### COMMENT

Considering the abundance of these two species on local rats in various areas and the lack of any reports of scrub-itch due to mite attack, it does not appear likely that either of these species is a significant human parasite. On the other hand, there is no evidence to indicate that species of *Neoschongastia* are not susceptible of infection with *Rickettsia orientalis*, and it is possible that under the local conditions in Mindoro, either of the present species could contribute to natural maintainance of this agent among local rats in focal areas. Under the conditions of the campaign there were neither the laboratory facilities nor the time to test adequate samples of mites for infection during the present study.

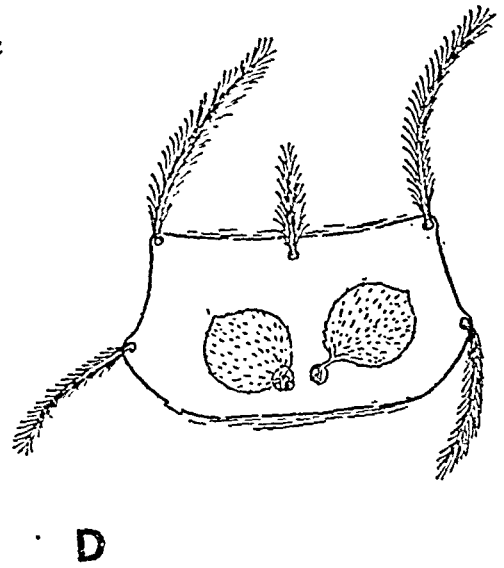
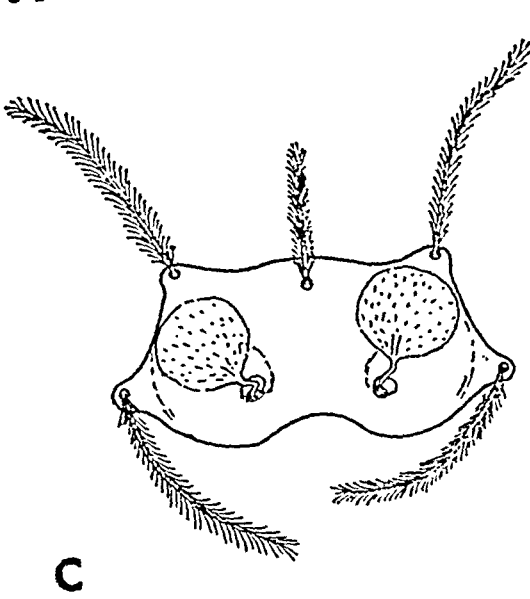
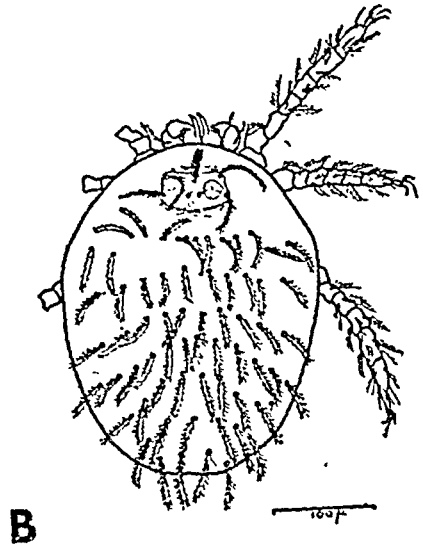
#### SUMMARY

Two new species of parasitic mites of the genus *Neoschongastia*, family Trombiculidae, namely, *N. philippensis* n.sp. and *N. kohlsi* n.sp. are described in this article. Both were taken from the ears of field rats, *Rattus mindanensis mindanensis* and *R. vigoratus*, indigenous to a focal area of scrub typhus in the vicinity of San Jose, Mindoro, Philippine Islands. The type specimens are deposited in the collections of the U. S. National Museum.

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PLATE I. A. Dorsal view of *Neoschongastia philippensis*; B. Dorsal view of *N. kohlsi*; C. Scutum of *N. philippensis*; D. Scutum of *N. kohlsi*







# CLINICAL AND LABORATORY VARIATION OF VIRULENCE IN SCRUB TYPHUS

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## INTRODUCTION

During the past twenty years numerous investigators have reported clinical and laboratory data on scrub typhus in Malaya, Sumatra, New Guinea and Australia. These descriptions have been similar in many essential details. The mortality has varied between 2 and 10 per cent and since 1942 a similar case fatality rate has been observed in the Australian and American armed forces (1).

The variation in severity, as reflected by the mortality rate, has been attributed to a number of factors. The general physical condition of the patients, the length of illness and the mode and duration of evacuation prior to hospital admission, the patient's age particularly if over forty years and the presence of intercurrent infections such as malaria and dysentery all seem to play a rôle. Although it is well known that variations in virulence of the *Rickettsia* of Rocky Mountain Spotted Fever are reflected in the severity and mortality of that disease (2), there is no recorded evidence available indicating a similar variation in *Rickettsia orientalis* Nagayo et al, the causative agent of scrub typhus (Tsutsugamushi disease).

In a recent large outbreak of scrub typhus, in which 1255 cases were studied, the average case was milder than in previous outbreaks in American troops. The mortality rate was only 0.6 percent although the previously given factors mentioned above which influence the severity seemed much the same.

The purpose of this report is to show a correlation between the variation in severity of clinical cases and the variation in virulence of corresponding strains of *Rickettsia orientalis*, as observed in laboratory animals.

## CLINICAL DATA

The clinical data used in this study were gathered in three different localities in the SWPA. In a previous report (3) the environmental and epidemiological features of these three foci, designated as A, C, and D, as affecting the armed forces, were discussed. Only the pertinent clinical facts are included here, as detailed data are recorded elsewhere (4, 5).

The cases of scrub typhus observed in area A were similar in most respects to those seen in other geographical locations in New Guinea, both in civilians and in the armed forces. There were 32 deaths in 449 cases or a mortality of 7.1 per cent. This rate falls within the usual accepted limits given by numerous investigators. Nearly all these cases were classed as moderately severe to severe.

In a group of 74 cases of scrub typhus in area A, the average duration of fever in patients who recovered was 18 days. The minimum duration of fever in

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proven cases was 11 days. In those cases with complications, particularly secondary bronchopneumonia due to pyogenic organisms, the duration of fever reached a maximum of 27 days. Since the minimum duration of fever was 11 days and since all cases showed moderate to severe degrees of prostration, no case in this group was considered as mild. Temperature readings of 104°F. were frequently seen and 105°F. and 106°F. were not uncommon. The fever curve was a high remittent type and fell to normal by lysis of a few days duration.

Signs of moderate to severe cardio-respiratory involvement were seen in two-thirds of these cases. During the first few days of illness the pulse rate was relatively slow in relation to the height of fever, but after this it tended to follow the fever level, particularly in the second and later weeks of the disease. Cardiac irregularities, fall in blood pressure and a rapid bounding or dicrotic pulse were also noted. The respiratory rate was elevated, frequently to 40 or more respirations a minute, and was associated with dyspnea. On examination of the lungs, there were varying degrees of dullness over the lung bases and numerous medium and coarser râles. Cyanosis of a mild to moderate degree was also noted.

Eschars, the initial lesion at the site of attachment of the infected mite, occurred in 89 per cent of 74 cases. Since Lewthwaite and Savor (6) have shown immunological identity between the two diseases previously differentiated only on the basis of the presence or absence of a primary eschar, the 11 per cent of cases without eschars cannot be justifiably rejected as not being scrub typhus. Associated with the eschars there was regional lymphadenopathy and generalized enlargement of the lymph nodes was noted in all cases.

Signs of neurological involvement consisting of acute delirium, other mental change and peripheral or eighth nerve neuritis were seen in 23 per cent of the total.

In the group of 74 cases, Weil-Felix tests were carried out on blood serum samples collected shortly after admission and subsequently every 3 to 5 days during the critical serological period when the initial rise in Weil-Felix titre took place. The technique of the test was recommended by the National Institute of Health (7). A test was considered positive when there was 75 per cent or more agglutination of 0.1 per cent formalized *Proteus* OXK antigen in a serum dilution of 1:20 or higher following gentle agitation of the tube. No false reaction was observed in over 600 tests. Appropriate parallel controls using *Proteus* OX19 and *Proteus* OX2 antigens showed no significant rise in titre.

The average initial rise in *Proteus* OXK titre occurred on the sixteenth day of illness with extremes ranging between the tenth and twentieth-fourth days of disease. However, since approximately 9 per cent of patients recovering from scrub typhus never developed agglutinins for *Proteus* OXK, the time at which this test became positive could only be used as a relative criterion for judging the severity of illness and recovery.

The relative mildness of the majority of 1255 cases of scrub typhus observed in area D was in marked contrast to the usual severity observed in other areas.

Of this group there were only 8 deaths, or a mortality of 0.6 per cent. The clinical picture in most of the cases was surprisingly mild. Indeed the diagnosis of scrub typhus was difficult to make in those cases of a few days' duration which showed minimal signs and symptoms. Many showed only protracted weakness and a rapid pulse rate following a short febrile illness.

In a group of 485 patients with complete fever charts, the average duration of fever was 14.6 days as contrasted to 18 days in the group A patients. With the limited facilities of a crowded evacuation hospital in a forward area, complete

TABLE 1  
*Clinical data on scrub typhus patients*

	AREA A	AREA C	AREA D
Number of cases.....	449	26	1255
Deaths.....	32	2	8
Mortality in per cent.....	7.1	8	0.6
Eschars in per cent.....	89 (74 cases)		51
Severe cardio-respiratory involvement in per cent.....	66 (74 cases)		<2
Neurological involvement in per cent.....	23 (74 cases)		<2
Average duration of fever in days.....	18 (74 cases)		14.6 (485 cases)
Average day of initial Weil-Felix.....	16 (229 tests)		10 (668 tests)

TABLE 2  
*Summary of Weil-Felix reactions in areas A and D*

DAY OF DISEASE	3	5	7	9	11	13	15	17	19	21	23	25
Area A No. of tests.....	4	14	18	25	26	25	28	25	21	17	15	15
No. of positive.....	0	0	0	0	4	7	12	16	16	15	12	10
Per cent positive.....	0	0	0	0	15	28	42	64	76	88	80	67
Area D No. of tests.....	19	60	76	71	113	74	81	66	45	25	22	16
No. of positive.....	4	12	20	31	70	57	65	52	39	21	14	13
Per cent positive.....	21	20	24	44	62	77	80	79	87	84	64	81

fever charts were not kept on the remainder of patients. Most of these were less acutely ill and it was certain that the average duration of fever for the whole group would have been shorter if this group of 770 patients had been included.

Eschars were looked for carefully in each case, but were observed less frequently in area D, 51 per cent of cases, than in area A. Lymphadenopathy was present in 97 per cent of all cases. Severe cardio-respiratory and neurological involvement were relatively infrequent, occurring in less than 2 per cent of cases, whereas it occurred in 66 per cent and 23 per cent of cases respectively in area A.

Coinciding with the relative mildness of the disease in Area D there was an earlier initial rise in the Weil-Felix titre. Of 155 tests carried out in the first

week of illness 23 per cent were positive while in area A, of 36 tests during a corresponding period, none was positive. The average initial rise occurred on the tenth day which was 6 days earlier than in the cases in area A.

From area C a small group of 26 cases with a mortality rate of 8 per cent is included. Only a relatively few people visited this area, but prior to anti-mite measures, over 60 per cent of all personnel living any length of time on the island contracted scrub typhus. Highly virulent strains of *Rickettsia orientalis* were isolated here. Unfortunately, clinical data on patients in this outbreak is not available.

Tables 1 and 2 summarize the pertinent clinical and serological data on the patients studied. In the group of patients from area D the lower mortality rate, lack of severe clinical manifestations, shorter duration of illness and earlier rise in initial Weil-Felix titre is apparent.

#### RICKETTSIA ORIENTALIS IN LABORATORY ANIMALS

In Sumatra, Dinger, and Wolff and Kouwenaar (8) found that infected material from scrub typhus patients injected intraperitoneally in white mice caused death of these animals in an average of 10 to 12 days in almost all (approaching 100 per cent) animals. Constant gross anatomical and microscopic changes were noted and smears of the characteristic sticky peritoneal exudate and peritoneal scrapings stained by the Giemsa method showed rickettsiae morphologically similar to those of Tsutsugamushi fever. Because of their high susceptibility to infection white mice have been used as the laboratory animal of choice by numerous investigators and by this means the etiological agent of scrub typhus in American troops has been shown to be *Rickettsia orientalis* (9).

In area A in 32 consecutive attempts 31 strains of rickettsiae were isolated from blood of patients with or dying of scrub typhus. Fifteen of these strains were from patients' bloods taken during the third to the tenth days of febrile illness. The remaining 16 strains were isolated in 17 attempts from heart's blood at autopsy. Each strain was isolated in a similar manner by grinding a small portion of blood clot aseptically with sand, adding a small amount of physiological saline, centrifuging at slow speed and injecting 0.3 cc. of the supernatant fluid intraperitoneally into each of a group of 3 white mice. The mice so infected died in 10 to 18 days, and showed characteristic gross anatomical changes. Smears of the peritoneal exudate, differentiated with Giemsa's stain, confirmed the presence of the small intra and extracellular diplococcal bodies characteristic of *Rickettsia*. The one failure to isolate a strain of *Rickettsia* in 32 consecutive attempts was from an autopsy case dead 10 hours before post mortem examination.

Following initial isolation strains were passed 2 to 5 times in successive groups of 3 or 4 mice each in order to confirm the initial observations and to observe the nature of reaction in mice. All passages were made by intraperitoneal injection of 0.3 cc. of a 1:10 dilution of 0.85 per cent saline peritoneal washings from infected mice. It would have been interesting to have carried all strains through a large series of passages but limited animal supply prevented this. However,

during the periods of observation, no essential difference from the strains carried over a period of months was seen.

The A No. 1 strain was isolated initially by the USA Typhus Commission from a typical case of scrub typhus in area A and was maintained by serial passage of infected peritoneal fluid in groups of 3 or 4 mice during a period of 15 months involving 64 passages. During this entire time the *Rickettsia* killed infected white mice uniformly in an average of 7 days. The occasional early death of a mouse was balanced by those that survived slightly longer than the average. Very rarely a mouse overcame the infection. An additional strain A No. 2 isolated in area A, was carried over 4½ months in groups of 4 mice through 18 passages. Again the mice died on an average of 7 days following infection and this strain showed no essential difference from the A No. 1 strain.

In the small, highly endemic area C, 3 strains of *Rickettsia* in 3 attempts, were isolated from mites removed from rats (3). Additional attempts were invalidated because of intercurrent bacterial infection which killed the mice short of the necessary time for rickettsial infection to be determined. These 3 strains proved highly virulent for mice. In 14 serial passages in groups of 4 mice death occurred on an average of 6 days following infection. One of these 3 strains C No. 11, was carried further over a 7 month period involving 35 passages. Again the mice died on an average of 6 days following intraperitoneal injection of infected material.

In area D the bloods from 8 clear cut cases of scrub typhus were inoculated into groups of 3 mice each. All cases were febrile at the time of mouse inoculation, but the duration of illness at that time ranged from 2 to 16 days. Primary eschars were present in 4 while the site of attachment of the infected mite could not be found in the other 4. The total duration of fever varied from 9 to 21 days, although the exact time could not be determined in 3 cases because of evacuation prior to defervescence.

All 8 groups of mice became infected with *Rickettsia orientalis*. However, only 4 strains, D No. 1, D No. 2, D No. 3, and D No. 4, were sufficiently virulent to kill the infected mice. The remaining 4 strains, D No. 5, D No. 6, D No. 7, and D No. 8, produced an inapparent type of infection. None of these latter groups of mice appeared ill during the course of infection and none died. All but one mouse of the groups showing an inapparent infection survived a challenge dose of 1000 MLD of the virulent strain C No. 11 isolated in area C. Four control mice inoculated at the same time died of rickettsial infection. The challenge dose was given one month following the initial blood infection and was repeated using 4 controls one month later with similar survival. The mouse that failed to survive the challenge dose was initially inoculated with blood drawn from patient D No. 8 on the sixteenth day of illness. It was assumed that blood taken late in the course of scrub typhus was non-infectious for this animal although the other 2 in the group apparently became infected and developed immunity.

Table 3 gives data on the 8 scrub typhus patients studied for *Rickettsia orientalis*. Group I includes those 4 patients infected with *Rickettsia* sufficiently virulent to produce death in the mice initially following intraperitoneal injection

of blood. Group II includes those 4 patients infected with *Rickettsia* of only sufficient virulence to produce an inapparent infection in white mice, as proven later by survival following a 1000 MLD challenge dose of a virulent strain, C No. 11. From a clinical standpoint, the two groups were similar; they all appeared moderately ill. Two in each group showed eschars and 2 did not. The day of disease on which the blood was taken roughly corresponded in the 2 groups and the total duration of fever was approximately parallel as nearly as could be determined.

The 4 strains isolated in area D were carried by serial passage of infected peritoneal fluid during a period of 5 months in a manner similar to that used for the other strains. Probably because of the prolonged duration of illness in mice, troublesome bacterial infection was encountered, particularly in the D No. 3

TABLE 3  
Data on eight patients studied for *Rickettsia orientalis* in area D

PATIENT	ESCHAR	DAY OF DISEASE BLOOD TAKEN	DURATION OF FEVER
Group I. <i>Rickettsia</i> isolated			
			days
D No. 1	+	2	10+
D No. 2	+	6	13
D No. 3	0	8	12
D No. 4	0	11	14+
Group II. <i>Rickettsia</i> not isolated: mice immune			
D No. 5	+	4	9
D No. 6	+	6	
D No. 7	0	10	17
D No. 8	0	16	21

strain. This necessitated passage of brain tissue, ground up with sulfathiazole crystals or penicillin, depending on the type of bacteria, until such contaminant was eliminated. No contaminated passage was used in calculating the average duration of rickettsial infection.

Although there was some individual variation between strains in the average day of death in mice following intraperitoneal infection, all strains averaged at least 2 days longer than those isolated in area A, and 3 days longer compared to those from area C. In the latter case this represented a 50 per cent or greater prolongation of survival time. In 13, 15, 9, and 15 passages, the average day of death was 9.5, 9.0, 9.1, and 10 days respectively.

To further demonstrate variation in virulence of strains from areas A, C, and D, titrations were made by diluting the standard passage material in physiological saline by integers of 10. Three-tenths cc. of each dilution was injected intraperitoneally in groups of 4 or 5 mice. The 50 per cent mortality,  $MLD_{50}$ , was calculated according to the method of Reed and Muench (10), and the titre expressed as the logarithm of the dilution. In calculating this end point all

deaths were considered to be due to *Rickettsia orientalis* when a peritoneal smear made at autopsy and stained with Giemsa's stain showed characteristic organisms in the cytoplasm of the large mononuclear cells.

The virulent C No. 11 strain from area C showed the highest dilution titre. The  $MLD_{50}$  was  $10^{-7.0}$  as determined by a titration using 24 mice. The A No. 1 strain showed an  $MLD_{50}$  titre of  $10^{-5.6}$ , combining the results of 4 titrations involving 83 mice. Two strains from area D showed  $MLD_{50}$  titres similar to the A No. 1 strain; in titrations using 39 mice each, the  $MLD_{50}$  titres were  $10^{-5.7}$  and  $10^{-5.8}$ . However the other 2 strains, in titrations using 20 and 32 mice, exhibited  $MLD_{50}$  titres of  $10^{-4.3}$  and  $10^{-4.6}$  respectively.

Although the  $MLD_{50}$  titres graded from  $10^{-4.3}$  to  $10^{-7.0}$ , the maximum differences were significant, as a variation of 2 or more log places represented a hundred fold or greater difference in dilution factor. Furthermore, it must be borne in mind that the 4 strains from area D used for titrations represented the more virulent ones involved in the outbreak. The less virulent strains produced only inapparent infections in the inoculated mice.

To determine whether the lower  $MLD_{50}$  titres observed were due to merely lack of infection, or to inapparent infections from which the mice recovered, many of those surviving were given challenge doses of 1000 MLD of strain C No. 11. Forty-six mice survived the titrations carried out on the 4 strains from area D. Of these, 41 were subjected to challenge doses of 1000 MLD and all survived. A total of 28 control mice received similar infectious doses and all died of rickettsial infection. This indicated that mice surviving the original titration doses underwent an inapparent type of infection with subsequent immunity.

When the minimal infectious dose of *Rickettsia* for the strains from area D was calculated by combining the number of mice proven to be infected at autopsy with those proven to be infected by survival of challenge doses of virulent *Rickettsia*, it was found to closely approximate the  $MLD_{50}$  titre for the virulent C No. 11 strain. Consequently it should be concluded that the differences in  $MLD_{50}$  titres observed between the various strains was on the basis of lethal virulence and not due to lack of infectiveness.

To determine the nature of immunity of the mice surviving challenge doses, the brains of a group of 4 mice initially infected 2 to 10 months previously and challenged one month previously were emulsified in saline and injected intraperitoneally into groups of 4 mice each. Members of all 4 groups died of rickettsial infection. This procedure was repeated on another group of 4 mice infected initially 3 months previously and challenged 2 months previously. Again all groups showed positive smears for *Rickettsia orientalis*. One of the strains isolated following intraperitoneal injection of emulsified brain, was carried for 5 passages and soon developed the characteristics of C No. 11, killing the mice in 6 days on the fourth and fifth passages. This was interpreted to indicate that the normal lack of resistance of mice was initially altered in some way so that they were able to tolerate an infection similar to the inapparent type seen in the wild rat which is a natural reservoir of scrub typhus. Table 4 summarizes the strain passages and titrations described.

In general laboratory workers have found that guinea pigs react in an irregular manner to intraperitoneal injections of *Rickettsia orientalis*. The fever curves frequently are non-specific and the mortality is low, varying from none to 50 per cent or more. In a study on about 1000 guinea pigs, Kouwenaar and Wolff (11) found a characteristic fever in 3 of 30 strains and varying mortality in these animals.

The strains of *Rickettsia* isolated in areas A, C, and D seemed to show differences in virulence when inoculated intraperitoneally into guinea pigs, although relatively small numbers of animals were used. The inoculum was the same in all animals; 0.5 cc. of a 1:10 saline dilution of infected mouse peritoneal washings. Rectal temperatures were taken twice daily and plotted in graphic form. There was marked normal variation, with temperature as high as 103.2°F. On death

TABLE 4

Summary of passages and titrations of strains of *Rickettsia orientalis* in white mice.

	PASSAGES			TITRATIONS	
	Period of observation	No. of passages	Aver. day of death	Number of mice used	MLD <sub>50</sub>
Area A					
A No. 1	15 mo.	64	7	83	10 <sup>-5.6</sup>
A No. 2	4.5 mo.	18	7		
Area C					
C No. 11	7 mo.	35	6	24	10 <sup>-7.0</sup>
Area D					
D No. 1	5 mo.	13	9.5	32	10 <sup>-4.6</sup>
D No. 2	5 mo.	15	9.0	39	10 <sup>-5.7</sup>
D No. 3	5 mo.	9	9.1	39	10 <sup>-5.8</sup>
D No. 4	5 mo.	15	10.0	20	10 <sup>-4.3</sup>

of the animal the gross anatomical changes were observed to be similar to those previously described (11), and Giemsa stained smears of the peritoneal exudate consistently revealed organisms characteristic of *Rickettsia*.

The strain isolated from area C produced the most severe reaction in guinea pigs. Of a group of 10 animals infected with C No. 11, 70 per cent showed fever above 104°F. and the mortality was 90 per cent. A No. 1 strain produced an intermediate type of reaction in a total of 20 animals observed. The fever curves were irregular with little or no pyrexia above 104°F. and there was 60 per cent fatality. The D No. 1 strain, isolated in area D, produced a milder type of reaction in guinea pigs. Again the fever curves were irregular, but of 10 animals inoculated, only 30 per cent died.

To show that the various strains studied were homologous in nature, varying only in virulence, without true heterology, cross immunity tests were carried out in a small number of rabbits according to the method of Lewthwaite and



Savor (6) as taken from Nagayo et al. Infective material, when injected into the anterior chamber of the eye, produced a characteristic irido-cyclitis following an incubation period. Subsequent to the initial infection, these rabbits were immune to intraocular injection of an homologous strain in the opposite eye.

Typical ocular reactions were demonstrated in 6 rabbits infected with the A No. 1 strain. The incubation period averaged 4.5 days and the acute reaction lasted on an average of 20 days. The D No. 1, D No. 2, and D No. 4 strains also produced satisfactory ocular reactions, although milder in nature, in 3 rabbits. The incubation period was prolonged to an average of 9 days, while the length of the reaction averaged 12 days.

The D No. 1, D No. 2, and D No. 4 strains were injected intraocularly into groups of 2 immune rabbits, with one simultaneous control as indicated above.

TABLE 5  
*Rickettsia orientalis* in guinea pigs and rabbits

	AREA A	AREA C	AREA D
Reaction in guinea pigs following intraperitoneal injection			
No. of guinea pigs.....	20	10	10
Mortality in per cent.....	60	90	30
Fever above 104° F.....	Irregular	70 per cent	Irregular
Intraocular infection in rabbits			
Incubation period (days).....	4.5 (6 rabbits)		9 (3 rabbits)
Duration of reaction (days).....	20		12

In none of the 6 rabbits was there any ocular reaction due to *Rickettsia orientalis*. Thus the cross immunity of the strains isolated in areas A and D was indicated, although the A No. 1 strain produced a more severe reaction. Studies on the D No. 3 strain were invalidated because of secondary bacterial contamination. Table 5 summarizes the laboratory studies on guinea pigs and rabbits.

#### DISCUSSION

It is believed that the clinical data presented on the cases from area A represents an average picture of scrub typhus in the New Guinea area, although there are notable exceptions in small outbreaks. On the other hand the large percentage of mild cases in area D is unusual.

The known factors which influence the severity and mortality in patients appear to be similar in the different groups of troops observed. It cannot be said that the forces in area D were living or fighting under more adverse conditions than those in other areas. Indeed, 2 of the 8 fatal cases of scrub typhus in area D were complicated with malaria and dysentery.

Previous to the experience in area D it was thought that *Rickettsia orientalis* was almost constantly fatal for mice. In area D, 4 of the 8 strains failed entirely to kill groups of mice and, on further study, the other 4 somewhat more virulent

strains produced milder infections in laboratory animals than strains isolated in other outbreaks.

On the basis of these clinical and laboratory observations it can be stated that the variation in severity of attacks of scrub typhus may be ascribed in part to variation in the virulence of strains of *Rickettsia orientalis*. Such a variation is known to occur in other rickettsial diseases.

#### SUMMARY

1. Clinical data are presented on one small and 2 large outbreaks of scrub typhus which occurred in 3 different geographical locations in the SWPA and which showed variation in the severity of illness.

2. Representative strains of the causative agent, *Rickettsia orientalis* were isolated in white mice from cases in the 3 areas and produced typical reactions in white mice, guinea pigs and rabbits.

3. Strains isolated from cases in an outbreak with an extremely low mortality rate were less virulent for laboratory animals than strains isolated from cases in two other outbreaks with higher mortality rates.

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## EXPERIMENTAL STUDIES WITH BULLIS FEVER

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When Bullis fever was first reported as a distinct clinical entity by Woodland, McDowell and Richards (1), and as a disease having a rickettsia-like agent as its probable cause (2, 3), there was some question as to its identity with the other known arthropod-borne disease agents. There did not appear to be any relationship between the syndrome of Bullis fever and the agents of Q fever, typhus and Rocky Mountain spotted fever either by animal inoculations or by available serological procedures (4); however, there did appear to be some serological relationship between an agent isolated from a case of Bullis fever and a large number of convalescent cases of the disease. Blair and Bader (5) demonstrated the infectivity of guinea pig propagated strains of Bullis fever for humans. The Q fever agent has not been isolated by this laboratory from any case of Bullis fever, although many guinea pigs have been inoculated with blood specimens from natural cases of the latter disease. Fifty-six guinea pigs have been inoculated with tick emulsions of over 10,000 ticks (*Amblyomma americanum*), with individual pools ranging from 45 ticks to 1,409 ticks, and neither the Q fever nor the Rocky Mountain spotted fever agent could be detected in any of them. Parker and Steinhaus (6) report on the isolation of a filterable agent from rabbit ticks (*Haemophysalis leporis-palustris*) which were collected at Camp Bullis. No agent similar to this has been isolated in this laboratory from any of the human cases of Bullis fever, nor from any of the tick emulsions (*A. americanum*) which were inoculated into guinea pigs.

The Bullis fever syndrome is characterized by a sudden onset of severe frontal headache and fever; then leucopenia, lymphadenitis (regional or generalized), and generalized aches and pains are noted. The leucopenia exhibits a relative neutropenia and lymphocytosis. Occasionally a somewhat evanescent rash is observed; this might be called a skin flush by some observers. All of the cases have had a history of exposure to ticks (*Amblyomma americanum*); however, not all of the cases have been exposed to chiggers (*Eutrombicula alfreddugesi*).

This disease was described originally in men who had been exposed to ticks in Camp Bullis, Texas, whence came the name. At the time there was no logical reason why this disease should be confined in its incidence to the vicinity of Camp Bullis. The *Amblyomma americanum* tick is distributed rather generally from this part of Texas to the East and North and has been reported from the New

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England States (7). Several cases of illness clinically similar to Bullis fever have already been reported to us from East Texas.

A thorough ecological survey of the Camp Bullis Reservation by Brennan (8) revealed a multitude of ectoparasites. The principal ones affecting man were ticks (*A. americanum*) and chiggers (*E. alfreddugesi*). Mosquitoes, sandflies and reduviid insects were rarely observed. The reservation abounded in deer, jack rabbits and armadillos; in addition, there were some gray foxes, racoons, fox squirrels, opossums, ground squirrels and cotton rats.

#### EXPERIMENTAL STUDIES

In order to acquire some information as to the nature of the Bullis fever syndrome and its etiology, a number of experiments were performed in which human, male volunteers were inoculated with:

1. Whole blood from natural cases of the disease.
2. A chick embryo propagated agent which was derived from the blood of a natural human case of Bullis fever.
3. A chick embryo propagated agent which was derived from emulsions of ticks (*A. americanum*) which were collected from deer at Camp Bullis, Texas.
4. Bullis fever agents of both human and tick origins, to determine their immunological relationship.
5. Laboratory strains of Bullis fever to challenge the immunity of natural cases of the disease.
6. The agent of Colorado tick fever<sup>5</sup> to study the immunological relationship to Bullis fever.

A complete history was recorded from each individual volunteer; especially concerning his previous habitation and disease experience. No volunteer was selected who had ever lived in the vicinity of Camp Bullis, Texas, or who ever had an unexplained febrile illness, or who had any known experience with tick infestation. The volunteers were given a thorough physical examination and only those who were essentially normal were submitted to the procedures which are described below.

All of the inoculums which were employed were examined by culture on thio-glycollate medium, blood glucose cystine agar and blood plates and were all negative for detectable bacterial organisms.

#### *I. Whole blood transmission experiments*

Whole blood was collected aseptically from two typical naturally acquired cases of Bullis fever (W. C. and W. F. M.). The blood was collected immediately after admission to the hospital and was stored in the dry ice chamber for two days. The two specimens of blood were then thawed and injected (1.0 cc. subcutaneously), each into a healthy volunteer.

These men were subsequently examined at daily intervals with thorough physical and hematological studies (Chart II).

<sup>5</sup> We are indebted to Dr. E. R. Murgage, University of Colorado School of Medicine, Denver, Colorado, for his kindness in supplying us with hamster serum infected with the Colorado tick fever agent.

*Case I* (G. R.) was inoculated with blood from case W. C. and he began to feel ill on the fourth day. There developed a generalized lymphadenopathy, fever, a relative leucopenia, generalized aches and pains and a severe headache—all of the symptoms consonant with clinical Bullis fever infection. In addition, a marked generalized rash developed on the eighth day of illness and disappeared on the following day. Whole blood was collected from this case on the 4th day of illness and the serum from half of it was filtered through a Seitz E K filter pad.<sup>6</sup> The whole blood and the filtrate of its serum were thereupon each inoculated into one healthy volunteer, Cases IV and V.

CHART I

*Clinico-pathological reactions of ten cases of experimentally induced Bullis fever.*

CASE	AGE	INOCULUM	BLOOD COUNTS		FEV- ER	HEAD- ACHE	LYMPH- ADENO- PATHY	GEN. ACHES AND PAINS	RASH
			Orig.	Relative Leuko- penia					
I. G. R.....	21	Blood of W. C.	8,600	5,600	+	+	+	+	+
II. A. B.....	35	Chick Embryo (Human Strain)	9,700	4,600	+	+	+	+	—
III. J. M. K.....	21	Chick Embryo (Human Strain)	10,550	5,800	±	+	+	+	—
IV. B. M.....	25	Serum Filt. of I.	7,600	5,000	+	+	+	+	—
V. A. I.....	21	Blood of I.	10,900	6,600	±	+	+	+	—
VI. A. P. R.....	18	Blood of W F.M.	6,200	5,400	±	+	+	+	—
VII. J. G.....	18	Chick Embryo (Human Strain)	7,600	5,350	+	+	+	+	—
VIII. E. H.....	22	Chick Embryo (Human Strain)	7,700	5,250	+	+	+	+	—
IX. V. J.....	17	Chick Embryo (Tick Strain)	9,850	4,800	+	+	+	+	—
X. E. N.....	19	Chick Embryo (Tick Strain)	7,000	3,200	+	+	+	+	—
XI. C. S.....	21	Chick Embryo (Normal)	9,000	None	—	—	—	—	—

*Case IV* (B. M.). This volunteer was inoculated with Seitz E K filtrate of the serum of Case I—3.0 cc. subcutaneously. Six days after he was inoculated, this patient complained of severe headache, generalized aches and pains. He demonstrated a fever, enlarged lymph nodes, and a relative leucopenia. This discomfort persisted for six days and then recovery was spontaneous.

*Case V* (A. I.). This volunteer was inoculated with whole blood from Case I, 1.0 cc. subcutaneously. Eight days after inoculation, this patient began to complain of headache, generalized aches and pains, and he demonstrated a mild fever. His lymph nodes were enlarged and a reduction in white cell count could be traced. This condition persisted for seven days after which he recovered spontaneously (Chart II).

\* The filter pad employed in this experiment was not subsequently checked for accuracy.

Case VI (A. P. R.) was inoculated (1.0 cc. subcutaneously) with whole blood from W. F. M. The inoculum induced a mild febrile response and an insignificant relative leucopenia; however, he did manifest generalized lymphadenopathy, generalized aches and pains, and severe headache (Chart I).

Thus, the disease is transmissible to healthy volunteers via the blood of infected individuals, and is transmissible through more than one generation of transfers. The agent was filtered through a Seitz E K filter pad in one attempt.

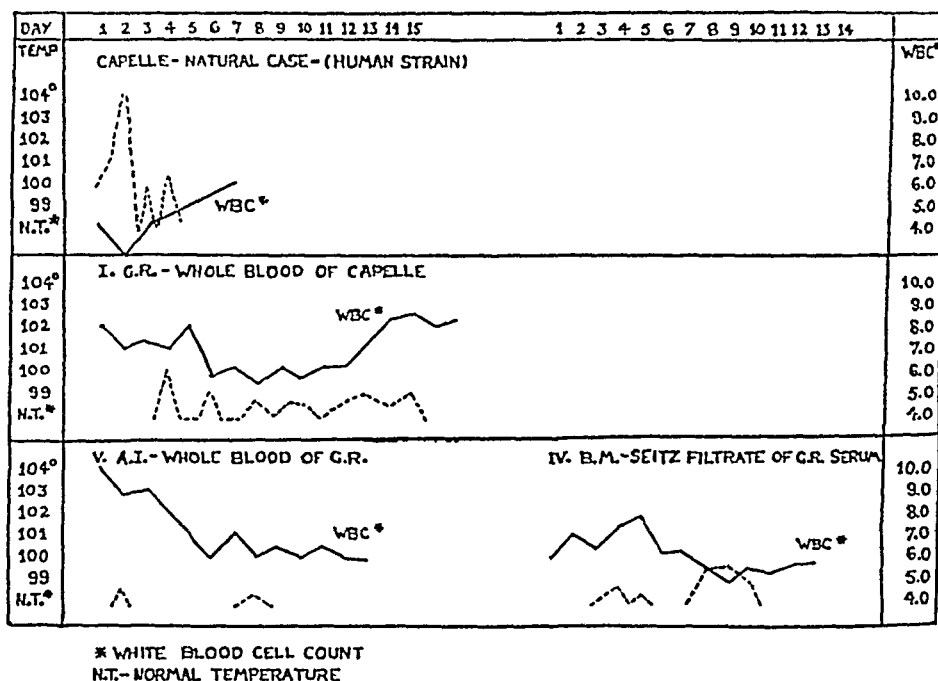


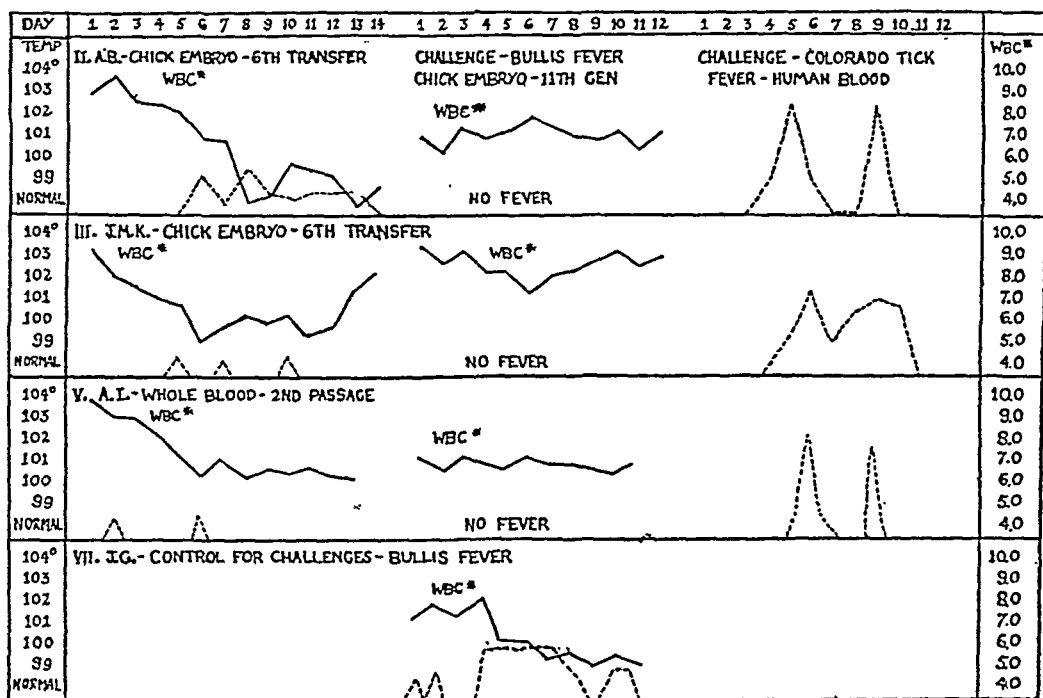
CHART II. Serial passage, Bullis fever

## II. Transmission of chick embryo propagated Bullis fever agent of human origin

Whole blood was collected from a febrile case of Bullis fever (J. G.) and it was inoculated into eight white mice, 0.25 cc. intraperitoneally. Nine days later the mice were destroyed and emulsions of their spleens were inoculated into eight additional mice. After eleven days the spleens from these latter mice were emulsified in alundum and saline and inoculated (0.5 cc.) into the yolk sacs of seven-day-old chick embryos. Subsequent serial passages of yolk sac emulsions were made into five or six-day-old chick embryos at six-day intervals and incubated at 35°C. The inoculums from all transfers were cultured in blood glucose cystine agar, thioglycollate broth and blood plates; in no instance was any bacterial agent detected. Smears were made of the yolk sacs and small cocco-bacillary bodies were observed, staining blue or red with the Machiavello stain and blue with the Giemsa stain. These bodies varied considerably in concentration, ranging from very numerous to relatively scarce in numbers. They were not observed to be intracellular.

The yolk sacs from the sixth passage of this human strain were emulsified with alundum and saline and were then inoculated into the culture media listed above. The remainder of the inoculum was stored in the dry ice chamber. No detectable bacterial growth was observed after five days; so the inoculum was thawed and 1.0 cc. of it was inoculated subcutaneously into each of two healthy volunteers.

*Case II* (A. B.). (Chart I) No local reaction ensued at the point of inoculation. Six days after inoculation this patient developed a severe frontal headache, generalized malaise and a progressive leucopenia (Chart III). This condition persisted for eight days and recovery was spontaneous.



\* WHITE BLOOD CELL COUNTS

CHART III. Challenge experiments with experimental cases of Bullis fever

*Case III* (J. M. K.). No local reaction developed at the point of inoculation. Four days after the inoculation was made this patient started to complain of generalized discomfort, headache, and a mild fever was noted. The superficial lymph nodes became generally enlarged and a relative leucopenia could be traced (Chart III). This patient remained ill for six days and recovered spontaneously.

*Case VII* (J. G.). After the agent had been passaged through eleven generations in chick embryos, a yolk sac emulsion of this passage was inoculated into one healthy volunteer (1.0 cc. subcutaneously). There was no local reaction at the point of inoculation. Four days after inoculation, this patient complained of a severe headache, generalized malaise and fever (Chart III). This was followed on the next day by generalized lymphadenopathy and a leucopenia. The illness persisted for seven days and recovery was spontaneous.

*Case VIII (E. H.).* After the agent had been passed through twenty series of chick embryos, the yolk sacs were emulsified and 1.0 cc. was inoculated subcutaneously into a volunteer. There was no subsequent local reaction. Four days after inoculation, this patient complained of having generalized aches and pains. The lymph nodes were enlarged. The patient was febrile and developed a leucopenia (Chart IV) and lymphocytosis. This illness persisted for eight days and recovery was spontaneous.

*Case XI (C. S.).* A control inoculation was made using a yolk sac emulsion from normal twelve-day-old chick embryos. The volunteer was inoculated with 1.0 cc. subcutaneously. There was no local reaction at the point of inoculation, and

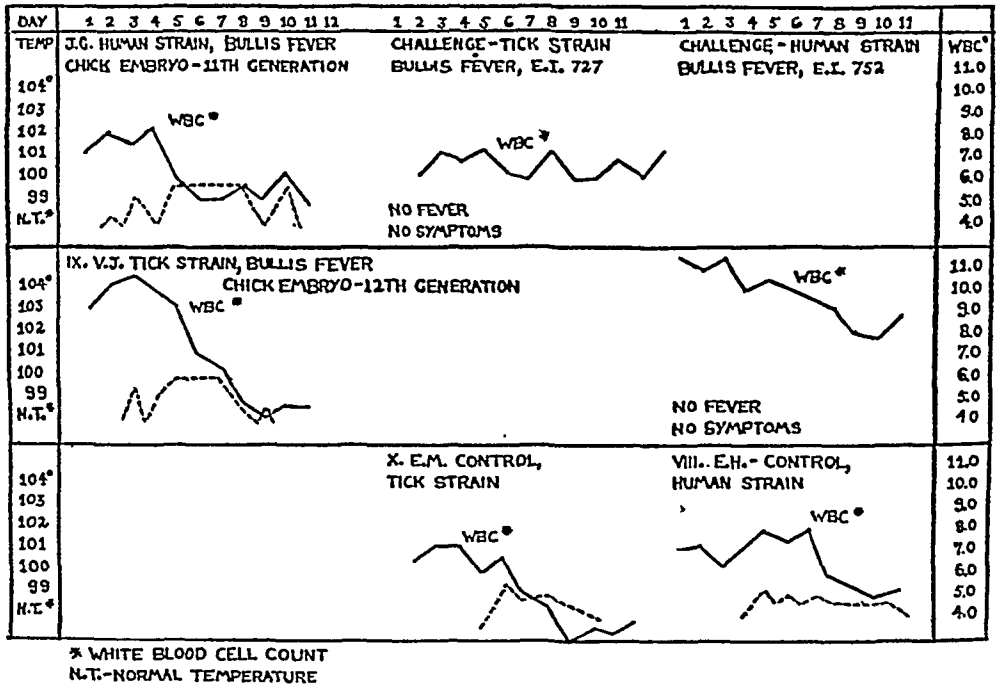


CHART IV. The cross relationship between Bullis fever strains of human and tick origin neither fever nor symptoms, nor leucopenia, developed subsequent to the inoculation (Chart V).

Thus, an agent, which originated from the blood of a natural case of Bullis fever, was propagated on the yolk sac of the developing chick embryo through twenty serial passages, and was still infective for human volunteers after these passages. A normal yolk sac emulsion failed to induce any reaction.

### III. Transmission of chick embryo propagated Bullis fever agent of tick origin

Three hundred and thirty-seven ticks (*A. americanum*) were collected from a deer which was shot in the Camp Bullis Reservation. The ticks were killed by freezing in a deep freeze ( $-10^{\circ}\text{C}.$ ); they were then washed three times with sterile saline, emulsified with alundum and saline and then inoculated into white mice (0.5 cc.) by the intraperitoneal route. After ten days, the mice were destroyed by ether and emulsions of their spleens were transferred to eight mice by the



intraperitoneal route. These latter mice were then destroyed after ten days and their spleens were emulsified and inoculated into the yolk sacs of six-day-old chick embryos. Yolk sacs were transferred in series every six days for twelve generations. The chick embryos were incubated at 35°C. after inoculation. There was no increase in virulence of this agent for chick embryos, as few of them died after inoculation. Smears of the yolk sacs demonstrated varying concentrations of small cocco-bacillary bodies which were similar morphologically to those bodies which were observed in the chick embryo yolk sacs with the Bullis fever strain of human origin.

*Case IX (V. J.).* One healthy volunteer was inoculated with a yolk sac emulsion of twelfth-generation tick strain Bullis fever—1.0 cc. subcutaneously. This patient subsequently developed fever, headache, generalized aches and pains,

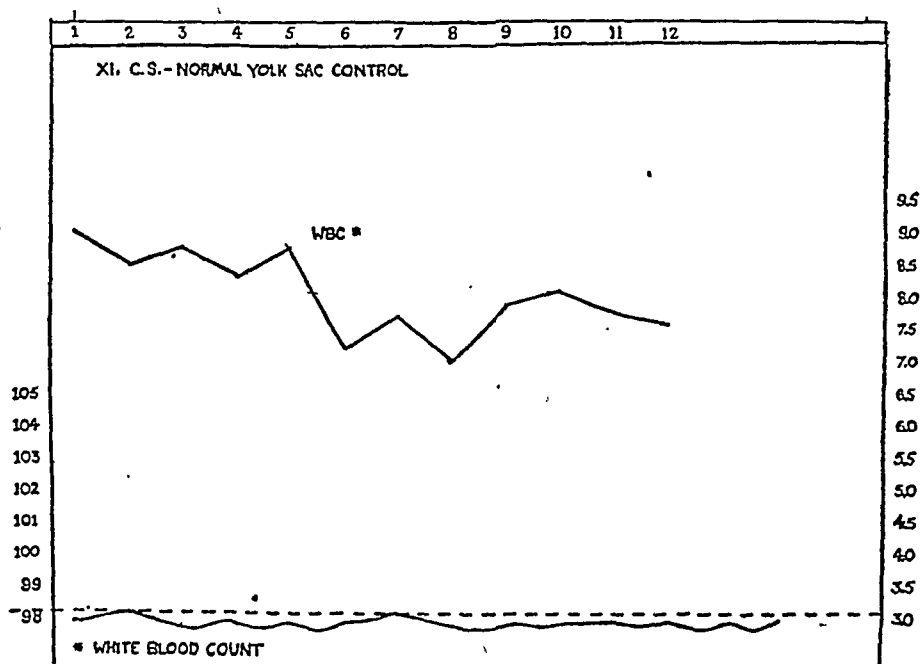


CHART V. Normal yolk sac control

lymphadenopathy, and relative leucopenia on the third day after the inoculation. This condition persisted for seven days and recovery was spontaneous (Chart IV).

*Case X (E. N.)* was inoculated with a yolk sac emulsion of fourth-generation tick strain Bullis fever, 1.0 cc. subcutaneously. This inoculum had been stored in the dry ice chamber for 39 days before inoculation. There was no subsequent reaction at the point of inoculation. Five days after inoculation, this patient started to complain of generalized discomfort and severe headache. He was febrile and the superficial lymph nodes were enlarged. A leucopenia and lymphocytosis was traced during the course of the disease (Chart IV). The illness persisted for five days and then was followed by complete recovery.

Thus, an agent which originated in ticks (*A. americanum*) from Camp Bullis was propagated in the yolk sac of the developing chick embryo through twelve serial passages, and was still infective for human volunteers after this sequence of

passages. The clinical syndrome which was induced by this agent was similar to that which was induced by the human strain.

*IV. The immunological relationship between the Bullis fever agents of human and of tick origin*

After one month of convalescence from infection with the chick embryo propagated *human* strain of Bullis fever, one patient (Case VII, J. G.) was challenged with the tick strain of Bullis fever agent and a new volunteer (Case X, E. N.) was inoculated as a control. Each person was inoculated with 1.0 cc. of a yolk sac emulsion of the agent. Subsequent examinations of these two men showed that the challenged individual remained unaffected while the control developed a typical Bullis fever syndrome (Charts IV and I).

After one month of convalescence from infection with the chick embryo propagated *tick* strain of Bullis fever, one patient (Case IX, V. J.) and a new control volunteer (Case VIII, E. H.) were each inoculated subcutaneously with 1.0 cc. of the chick embryo propagated human strain of Bullis fever. Subsequent examinations of these two men revealed that the challenged individual remained unaffected, while the control developed a typical Bullis fever syndrome (Chart IV).

Thus, the tick strain and the human strain of Bullis fever agents both induced a similar and typical Bullis fever syndrome in the inoculated susceptible individuals. They immunized against each other showing that the two strains are the same agent.

*V. Challenge experiments of naturally acquired cases of Bullis fever with laboratory propagated strains of the agent*

Two men (J. G. and W. F. M.) who had contracted Bullis fever while visiting at Camp Bullis, Texas, volunteered to undergo a series of challenge experiments. These two cases originally manifested fever, leucopenia, generalized malaise, lymphadenopathy and both had a history of tick bites while at Camp Bullis. A strain of Bullis fever agent was isolated from one case (J. G.), which is reported in experiment II above.

About one month after recovery from Bullis fever, each man was challenged with the chick embryo propagated human strain of Bullis fever—1.0 cc. of yolk sac emulsion subcutaneously. Daily examination of these men failed to detect any deleterious effect from this challenge experiment (Chart VI); however, the control for this inoculum (Case VII, J. G.) developed a typical infection with relative leucopenia, lymphadenopathy, generalized aches and pains, headache and fever (Chart VI).

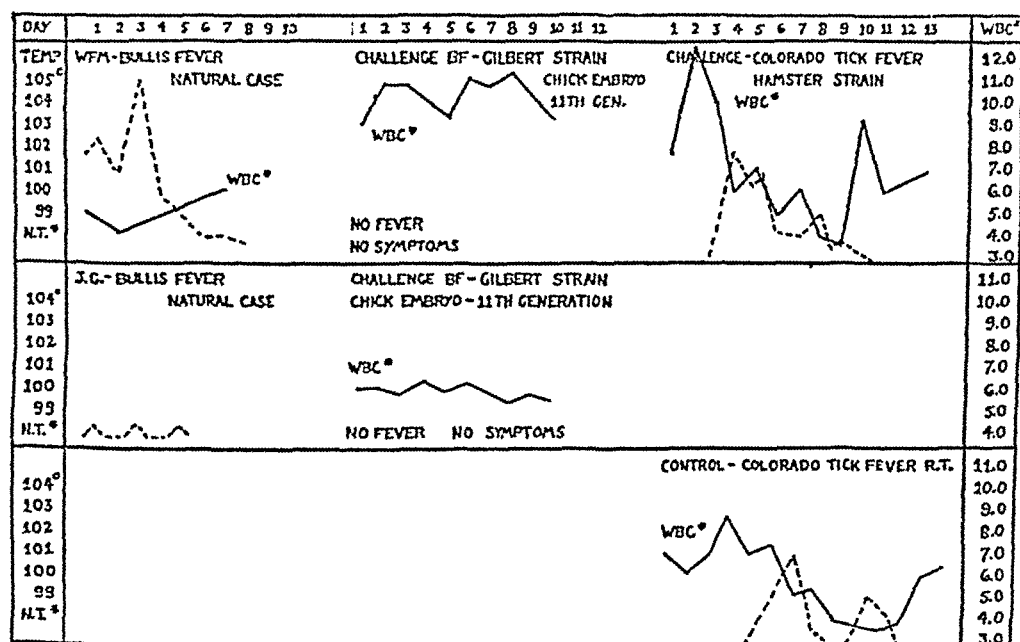
Thus, convalescent cases of the naturally acquired disease are immune to the chick embryo propagated strain of Bullis fever of human origin.

*VI. Immunity experiments with Colorado tick fever*

Two men, who had developed the Bullis fever syndrome after being inoculated with a yolk sac emulsion of human strain Bullis fever (Cases II and III), and one

man, with the second generation human blood transmission (Case V), were challenged with a yolk sac emulsion of human Bullis fever strain (1.0 cc. subcutaneously). A new volunteer (Case VII, J. G.) was also inoculated with this material for control purposes. All of the challenge experiments showed immunity to the disease agent and the control volunteer developed the Bullis fever syndrome (Chart III).

Three weeks after the Bullis fever challenge inoculations described above, each volunteer was inoculated with Colorado tick fever infected human blood. All of them developed the latter disease with generalized aches and pains, characteristic fever curve and severe headache (Chart III).



\* WHITE BLOOD CELL COUNT  
N.T.-NORMAL TEMPERATURE

CHART VI. Challenge experiments with natural cases of Bullis fever

One patient (W. F. M.) who had recovered from a naturally acquired infection with Bullis fever and who subsequently demonstrated immunity to the laboratory propagated human strain of Bullis fever was also challenged with Colorado tick fever infected hamster serum. This patient developed a typical case (9) of Colorado tick fever, with leucopenia, severe headache, generalized aches and pains, and high fever (Chart VI).

Thus, immunity is demonstrated between the Bullis fever agent which was transmitted with whole blood and the yolk sac propagated agent of human origin. There is no relationship between the human strains of Bullis fever and the Colorado tick fever agent.

#### DISCUSSION

On the basis of the information herein reported, it is established that Bullis fever is a transmissible clinical syndrome which can be reproduced by transferring

blood from a case of the disease to a normal individual, and that it can be maintained in series.

An agent which originated from the blood of a natural case of Bullis fever was propagated through twenty generations of chick embryo passages and its presence was then demonstrated by reproducing the disease in human volunteers by subcutaneous inoculation. This chick embryo propagated agent induced a disease of somewhat diminished severity when compared to the natural cases of the disease. This might be due to increased adaptation of the agent to avian tissues, although the virulence for chick embryos was not increased appreciably through repeated passages. Since the agent can be propagated in the chick embryo, an immunizing agent might be developed for future control of the disease.

An agent which originated from ticks (*A. americanum*), which were collected from deer at Camp Bullis, Texas, was propagated in the yolk sac of the developing chick embryo for twelve generations and it then induced a disease in volunteers which was indistinguishable clinically from that of natural cases and that of the Bullis fever strain of human origin. The disease was not actually reproduced by tick bites but by an agent which originated in tick emulsions. It would have been more accurate if feeding ticks had reproduced the disease, since the agent might have been resident passively in the ticks. However, the natural cases of the disease have histories of tick bites.

The critical phase of the problem was to determine the relationship of the chick embryo propagated Bullis fever strain to convalescent naturally acquired cases of the disease. Two such cases were challenged with the Bullis fever infected chick embryo material and they were immune; the inoculated control developed the disease. Apparently, there is a distinct immunological relationship between the agent isolated and the agent responsible for the natural cases of the disease.

Since no relationship could be detected between Bullis fever and the other known rickettsial disease agents, attention was directed toward Colorado tick fever, a disease which appears to be tick-borne and which is characterized by a low white blood cell count. In recent reviews of reports on Bullis fever (10), there is expressed, parenthetically, by the reviewer the opinion that Bullis fever might be related to Colorado tick fever. Challenge experiments with the Colorado tick fever agent in immune Bullis fever volunteers, both naturally acquired and experimentally induced cases, failed to demonstrate any relationship between these two diseases. Since the agent was filterable on only one attempt, it would be premature to make any definitive classification of the agent without additional studies. The agent appears to approximate in size the elementary body agents such as ornithosis more than it does the typical rickettsiae. The small coccobacillary bodies which were observed microscopically were so variable in occurrence as to suggest that some improvements were indicated in the technique of propagation. These small coccobacillary bodies did not appear to be the natural inclusion bodies of the chick embryo yolk sacs as described by Pappenheimer (11), as they were not noted by us in the controls and they are reported to be relatively scarce in embryos which are less than twelve days old.

All of the volunteers were examined serologically prior to and subsequent to their respective inoculations. They were examined by the complement-fixation test for Murine typhus, Rocky Mountain spotted fever, Q fever and lymphogranuloma venereum. Agglutination tests were also made on these serums for *Proteus* OX<sub>19</sub>, *Proteus* OX<sub>2</sub> and for the heterophile antibody. All of these examinations were negative.

Since the agent of Bullis fever has been found to exert a low degree of virulence in the laboratory animals thus far employed, it seems that the most accurate method for determining the presence of the agent is by human inoculation. The

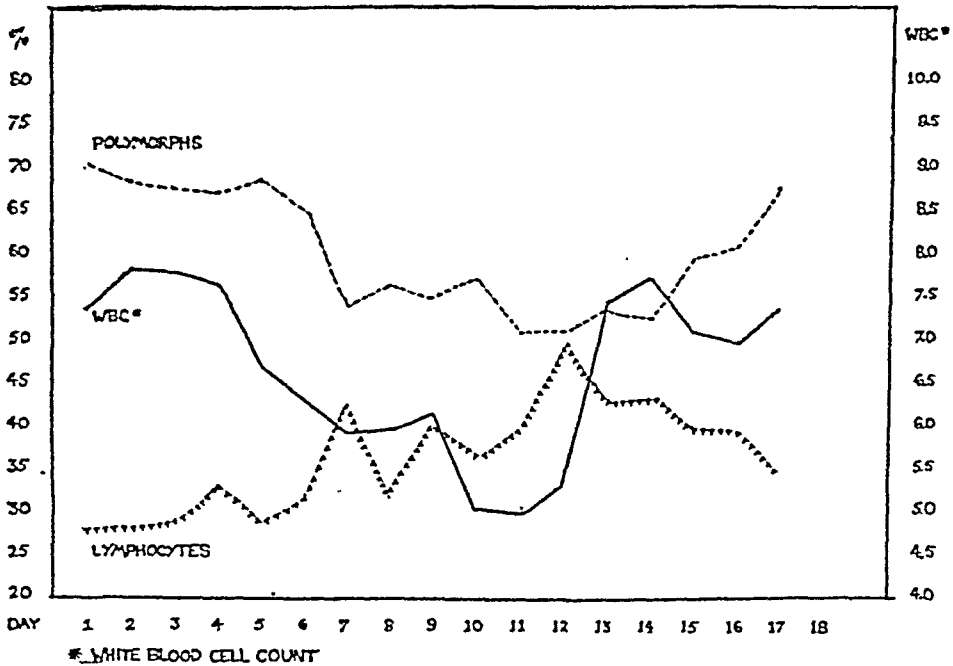


CHART VII. Average differential and total white blood cell counts, six cases experimental bullis fever

clinical syndrome of naturally acquired cases is rather clear; especially if associated with tick bites. The disease syndromes induced by the laboratory propagated human and tick strains of Bullis fever were indistinguishable from that of the naturally acquired infection.

The leucopenia reaches its lowest point at approximately eleven days after inoculation (Chart VII), and this point is generally followed by recovery of the patient. There was noted an increase in large mono-nuclear cells early in convalescence which were similar to the transitional type cells described in dengue; scrub typhus and other diseases which are characterized by a leucopenia. As the disease progressed in severity, many lymphoid cells were noted which contained minute cocco-bacillary bodies, in some instances somewhat similar to azurophilic bodies. In some instances these bodies were noted in the nuclear material as

well as the cytoplasm. They were not similar to the coccoid bodies which have been described in Colorado tick fever (9).

#### SUMMARY

From the results tabulated in this report the following conclusions may be made:

1. The Bullis fever syndrome is a distinct clinical entity which may be reproduced in humans by the inoculation of blood from febrile cases of the disease.

2. The Bullis fever agent from the blood of febrile cases has been propagated on the yolk sac of the developing chick embryo. The yolk sac propagated agent reproduced the Bullis fever syndrome in humans after twenty serial transfers in the yolk sac.

3. The Bullis fever agent has been isolated from tick emulsion (*A. americanum*) from Camp Bullis, and has been propagated in the yolk sac of the developing chick embryo. After the agent was propagated for twelve generations on the yolk sac of the developing chick embryo, it reproduced the Bullis fever syndrome in the human.

4. The immunological responses induced by natural cases of disease by the blood propagated yolk sac strain and by the tick propagated yolk sac strain are the same.

5. There is no immunological relationship between the agent of Bullis fever and the agent of Colorado tick fever.

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# A NEUROTROPIC VIRUS ISOLATED FROM *Aedes* MOSQUITOES CAUGHT IN THE SEMLIKI FOREST

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An outbreak of yellow fever occurred in Bwamba County Uganda in 1941, during which yellow fever virus was isolated from a human case and from *Aedes simpsoni* mosquitoes (1). Although that mosquito was clearly implicated as the vector in man, information obtained at that time and in earlier studies (2, 3) clearly pointed to an association of yellow fever with the forest. Since *Aedes simpsoni* occurs in the Bwamba forests only at the extreme edges, this indicated that it might not be the only insect involved in the epidemiological picture. An intensified field investigation was accordingly undertaken, one of the objectives being to learn whether other insects, especially those of the forest, might be implicated. Although mass immunization of the human population was meantime carried out, with resultant high incidence of immunity to yellow fever (4), the virus of that disease was again isolated from mosquitoes in 1942 and in 1944. Moreover 3 other viruses were isolated as well, one in September 1942 (5), a second in September 1943 and a third in April 1944 (Smithburn and Haddow, to be published). The purpose of the present communication is to record the isolation and describe some of the properties of the second of these, a neurotropic virus probably hitherto unknown.

The virus was isolated in September 1943 from a large lot of *Aedes* mosquitoes caught in an area in the Semliki Forest, known as Bunyamwera, hence the name provisionally adopted—Bunyamwera virus. A description of Bwamba County and of the Semliki Forest has already been written (6) so that it is necessary here only to discuss the mosquitoes from which the virus was isolated and to record the relevant facts about the locality where the insects were caught.

## BUNYAMWERA III AND THE MOSQUITO CATCH

There are 3 separate areas in Bwamba known as Bunyamwera. That with which we are concerned—Bunyamwera III—lies at the confluence of the Lamia and Semliki rivers and is uninhabited. The nearest legally inhabited area is about 6 miles to the south, but at the time of the mosquito catch in question, there was a small illegal fishing settlement of about 12 individuals across the Semliki River on the Congo shore. The area covered by the catch was about  $\frac{1}{2}$  square mile.

Flooding from the Semliki has killed off much of the hardwood timber of Bunyamwera and this has been replaced by a second growth of acacia, oil palm and wild date palm, though where the banks are high the primeval rain forest

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reaches the river. This forest consists mainly of the African ironwood (*Cynometra alexandri* C. H. Wright), a tree which in Bwamba forms a light, closed canopy at 50–80 feet. In such ironwood forest the undergrowth is very dense and forms a secondary canopy at 12–15 feet, in the shade of which mosquitoes of many species abound. During most of the year the ground is water logged and the numerous game paths form chains of muddy pools, well suited to the needs of ground-pool-breeding mosquitoes.

The Semliki River at Bunyamwera cannot be considered as a complete barrier to animal migration. Though it is deep and rapid and perhaps 150 yards wide, snakes have twice been seen to cross from the Congo shore. But the monkey fauna of the Congo bank comprises the grey or grivet monkey—*Cercopithecus aethiops centralis* Neumann—and the red-tailed guenon—*Cercopithecus nictitans mpangae* Matschie—while on the Uganda side the dominant species are the black-and-white colobus—*Colobus polykomos uellensis* Matschie—and the black mangabey—*Cercocobus albigena johnstoni* (Lydekker). This seems to indicate that primates, at least, do not cross the river. The other common animals of Bunyamwera are the red or forest buffalo, the elephant, the hippopotamus, the giant forest hog, the Uganda red hog, the harnessed bushbuck, the leopard and the crocodile.

The mosquito catch concerned was carried out on 5 successive days, 5,345 female mosquitoes being taken during this period. Of these, 5,133 were sent to the Institute at Entebbe. It has been our experience that in the Semliki Forest the majority of mosquitoes taken biting belong to the genus *Aedes*, while *Culex* spp. predominate in catches of resting mosquitoes in the undergrowth. Bunyamwera proved an exception, and *Aedes* spp. predominated in catches of both types.

The lot from which the virus was isolated consisted of 4,114 *Aedes*, belonging to 14 species, namely:

<i>Aedes</i> ( <i>Finlaya</i> ) <i>ingrami</i> Edw.....	1
<i>A.</i> ( <i>Stegomyia</i> ) <i>de-boeri</i> ssp. <i>de-meilloni</i> Edw.....	40
<i>A.</i> ( <i>Aedimorphus</i> ) <i>capensis</i> Edw.....	3
<i>A.</i> ( <i>A.</i> ) <i>simulans</i> N. and C.....	1
<i>A.</i> ( <i>A.</i> ) <i>argenteopunctatus</i> Theo.....	9
<i>A.</i> ( <i>A.</i> ) <i>mutilus</i> Edw.....	78
<i>A.</i> ( <i>A.</i> ) <i>domesticus</i> Theo.....	76
<i>A.</i> ( <i>A.</i> ) <i>tarsalis</i> Newst. and	
<i>A.</i> ( <i>A.</i> ) sp. n. near <i>A.</i> ( <i>A.</i> ) <i>abnormalis</i> Theo.....	1096
<i>A.</i> ( <i>A.</i> ) <i>cumminsi</i> Theo.....	225
<i>A.</i> ( <i>Banksinella</i> ) <i>circumluteolus</i> Theo.....	102
<i>A.</i> ( <i>B.</i> ) <i>palpalis</i> Newst. and	
<i>A.</i> ( <i>B.</i> ) <i>taeniarostris</i> Theo.....	2482
<i>A.</i> ( <i>Dunnus</i> ) <i>kummi</i> Edw.....	1

It will be noted that grouping has been resorted to in the case of one section of the subgenus *Aedimorphus* and one section of the subgenus *Banksinella*. In these groups the females are not reliably distinguishable in life. The *A. tarsalis* group predominated in the ironwood forest and the *Banksinella* spp. in the palm and acacia belt along the river.

It is not possible to say from which of the above-mentioned species the virus was isolated. One of 2 colobus monkeys shot at Bunyamwera during the catch proved to be immune to the virus. As the colobus is a markedly arboreal monkey this at first suggested a tree-haunting mosquito as the most likely to be involved. None of the *Aedes* listed above is markedly arboreal, with the possible exception of *A. ingrami*, but several of them have occasionally been taken in treetop catches. On the other hand, tests on 40 additional monkeys from Bwamba have failed to reveal any more immune specimens. The distribution of human immunity to the virus extends far into the open country of the Semliki plains north of the forest, and this seems to point to one of the *Banksinella* spp. as the most likely to be implicated in transmission, as the other *Aedes* spp. represented in the catch are, in Bwamba, confined almost entirely to forested areas.

The materials and methods employed in the laboratory and field investigations were the same as we have described in earlier studies (5, 7) and will be discussed here only as necessary in the individual experiments.

#### ISOLATION OF THE VIRUS

The 4,114 *Aedes* mosquitoes were received in the laboratory at Entebbe on the evening of September 19, 1943. On the following morning 15 of the insects were dead. These, together with the 4,099 living, which were killed with chloroform, were ground in a mortar with 30 ml. of 10 per cent normal human serum<sup>3</sup> and spun for half an hour in the angle centrifuge at about 3,000 r.p.m. A small portion of the supernate was passed through a Seitz EK pad (previously washed with serum-saline), and the filtrate was used for the intracerebral inoculation of a group of 6 mice.<sup>4</sup> The remaining unfiltered supernate—21 ml.—was inoculated subcutaneously into rhesus monkey No. 400.

The 6 mice receiving Seitz-filtered mosquito suspension remained well. Rhesus 400 appeared well during the first 24 hours but was found *in extremis* 44 hours after inoculation; it was then bled, and mice were inoculated intracerebrally with its serum. The monkey died while being bled. A suspension was made from a portion of its liver, and mice were inoculated intracerebrally with unfiltered and Seitz-filtered liver supernate. The remaining liver supernate (3.3 ml.), together with the remaining 2.4 ml. of serum, were inoculated subcutaneously into rhesus monkey 403.

Rhesus 403 had fever 24 and 30 hours after inoculation and was bled for subinoculation. Rhesus 415 received 1.5 ml. of Seitz-filtered serum subcutaneously and a group of 6 mice received Seitz-filtered serum intracerebrally; the filtration was done in order to eliminate any bacteria present, as it was thought that the

<sup>3</sup> One volume of serum to which was added 9 volumes of physiological saline—hereafter referred to as serum-saline. By normal human serum in this instance is meant a pool of sera from persons known to be non-immune to yellow fever virus. After the isolation of Bunyamwera virus and the discovery of methods for doing protection tests with it, all sera used for preparation of serum-saline diluent were first proved to be devoid of homologous antibody. Such diluents were invariably sterilized by filtration through Seitz EK pads prior to use.

<sup>4</sup> For these and other experimental procedures the animals were anesthetized with ether.

early febrile response of rhesus 403 might possibly have been due to a bacteremia. The latter animal recovered without showing any additional signs of illness.

The mice receiving the serum of the original monkey—rhesus 400—became ill or paralyzed beginning on the 5th day after inoculation. Two were sacrificed for passages and the remaining 4 died on the 6th, 7th, 8th and 9th days. Intracerebral subinoculations were successful, the incubation period being 4 days in the 2nd passage mice and 3 days in the 3rd. By the 5th passage the onset of illness was noticeable on the 2nd day. There were no surviving mice in any of these passage groups.

Mice receiving unfiltered liver suspension of rhesus 400 began to sicken on the 7th day, and those receiving filtered suspension showed signs on the 8th day. One of the former group survived and 3 of the latter, but intracerebral subinoculations from sick mice of each group were successful, with progressive diminution in incubation period similar to that seen in the series subinoculated from mice receiving the serum from rhesus 400. There were no survivors in either of these series after the 2nd mouse passage.

Mice receiving the Seitz-filtered serum of rhesus 403, taken at the time of fever 24 hours after inoculation, showed first signs of illness on the 4th day, and 2 further groups of mice were inoculated intracerebrally with suspensions of brains of these ill mice. All the original group were dead by the 8th day. One 2nd passage group began to sicken on the 4th day, and all were dead by the 8th day. The other 2nd passage group began to sicken on the 3rd day, and all were dead on the 5th day. By the 4th passage the incubation period was further reduced to 2 days. No "passage" mouse ever survived inoculation with this line of virus.

Thus 4 separate lines of virus were established in mice: 1 from serum, 1 from unfiltered liver suspension, 1 from Seitz-filtered liver suspension of rhesus 400 and the 4th from the Seitz-filtered serum of rhesus 403. Three of these lines were passed in duplicate for a time—a total of 7 lines being transferred in series 25 times. This was done because of differences in incubation periods and in potency, by reason of which we could not at first be certain that we were dealing with a single virus. However, after serial passage the incubation periods became the same with the 4 lines, and, as will be shown later, it was possible to prove their immunological identity. Thereafter only 2 lines were transmitted in series. The virus was transferred through 102 serial passages, with no mice surviving intracerebral inoculation of the supernate from 10 per cent brain suspension after the 2nd passage. By the 67th passage mice were so frequently dead on the 2nd day that it became routine to remove brains for transfer 24 hours after inoculation. Once the virus had become well adapted in mice and the initial decline in incubation period ended, the average survival time<sup>5</sup> was very slightly under 3 days (2.87 days for 477 mice of the 54th to 101st passages).

It is to be noted that the virus did not become established by direct passage from mosquitoes to mice, but was transmissible in mice after passage through a

<sup>5</sup> Mice alive 24 hours after inoculation and dead after 48 hours were considered as having survived 2 days; mice alive 48 hours after inoculation but dead after 72 hours were considered as having survived 3 days, etc.

rhesus monkey. It is also to be noted that it became adapted to mice more readily after 2 passages through rhesus monkeys than after but one. Unfortunately rhesus 400 succumbed, so that it was not possible to establish the origin of the virus from the mosquitoes by a change in the immune state of this monkey resulting from the inoculation of mosquitoes. However, the following points indicate that the virus did in fact originate from the mosquitoes:

1. It had a low original virulence for mice, with progressive adaptation after passage through monkeys.

2. Rhesus 400 had been under continuous observation for 13 weeks prior to inoculation of the *Aedes* mosquitoes in a room screened with 2 layers of monel metal gauze, 20 mesh to the inch, and during this time it showed no illness.

3. Other monkeys in the room at the same time, given the same diet and attended by the same persons showed no illness, but none of these received the mosquito suspension injected into rhesus 400.

4. Immunity to the virus has not been observed in any of our laboratory animals, indicating that it was not present in the mouse or monkey colonies at the time of isolation, before or since.

5. Illness of the type caused by Bunyamwera virus in mice has been observed only in mice receiving inoculations of the virus.

6. A colobus monkey, one of 2 shot in the catching area during the days the mosquitoes were being caught, was found to possess humoral antibody against the virus.

7. Humoral immunity against the virus has been found among the residents of Bwamba county.

It is therefore concluded that the virus was derived from the mosquitoes.

#### PATHOGENIC PROPERTIES OF THE VIRUS

*In White Mice.* Mice inoculated with Bunyamwera virus exhibit objective signs referable principally to the nervous system. Frequently the first sign is a state of hyperactivity and hyperreactivity. During this phase the coat is usually quite smooth; the animal, if momentarily quiet, responds to auditory or tactile stimuli by leaping vigorously or racing around its cage. Given freedom from confinement it runs madly in circles, always in one direction. At this and later stages slight stimulation may bring on convulsions from which the animal may not recover. Death, in fact, is frequently preceded by convulsions. During the hyperactive stage violent muscular spasms involving the head and neck are commonly seen. Such spasms may also involve one or more of the limbs.

If the animal survives the hyperreactive stage it soon passes into one of paralysis, early associated with coma. The paralysis involves all limbs, but more noticeably the hind ones. Convulsions occur also in this phase of the illness. In this stage the coat is roughened. Following intracerebral inoculation the entire period of objective illness usually does not exceed 24 hours, although it may be longer if the dose of virus inoculated is small. With 14th passage virus inoculated intracerebrally the mean survival time in mice receiving 1 in 100 filtered brain suspension (6,750 MLD) was 3.3. days; in mice receiving 1 in 100,000 filtered suspension (6 MLD) it was 6.5 days.

Bunyamwera virus is pathogenic for mice by intracerebral, intraperitoneal, intranasal and subcutaneous inoculation, as demonstrated by the following experiment.

Brains of 3 sick mice of the 21st passage group were ground in serum-saline to make a 10 per cent suspension. This was spun for 20 minutes at about 3,000

TABLE 1

Tests of pathogenicity of *Bunyamwera virus* by intracerebral, intraperitoneal, intranasal, and subcutaneous inoculations, showing variations in survival time

ROUTE INOCU- LATED	VIRUS DILU- TION, LOG	DEATHS OF MICE, DAYS AFTER INOCULATION															TREATY	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Died	Lost
I-C	3			4	3	4	1										12	0
	4			3	6	3											12	0
	5				4	5	3										12	0
	6				1	6	2	1	1								11	1
	7					2		2									4	8
	8								1								1	11
I-P	1			1	1	2	3	1	2	1			1				12	0
	2						2	6				1	1				10	2
	3					1	2	4	2				1				10	2
	4					4		4					1				9	3
	5							1	2	1							4	8
	6								2	1						1	4	8
	7							1	2								3	9
	8																0	12
I-N	1					4	6	2									12	0
	2						7	4	1								12	0
	3					2	2	4	3					1			12	0
	4						2	4		2	3	1					12	0
	5						2	3	3	1		3	1			P*	10	2
Sub- cu.	1					1	4	3	2								10	2
	2		2†			1	1	3	3	1							9	1
	3					2	4	3		1						M†	11	1
	4						1	4	2			1				M	9	3

\* Mouse showed specific paralytic symptoms.

† One died and another (injured) was destroyed. Neither is regarded in the test.

‡ Mice which were moribund. These are considered as having succumbed to the virus.

r.p.m. in the angle-head centrifuge; the supernate was removed and passed through a Seitz EK pad under 15 pounds air pressure. Serial decimal dilutions of filtrate were prepared with serum-saline diluent, and groups of 12 mice were inoculated intracerebrally, intraperitoneally, intranasally, or subcutaneously with various dilutions, the volume of inoculum being invariably 0.03 ml. End points were not obtained with the intranasal and subcutaneous inoculations, as

the highest dilutions were not injected by these routes. However, the results of the experiment, given in table 1, demonstrate the pathogenicity of the virus by various routes and the variation in survival time occasioned thereby.

Bunyamwera virus has been transmitted in series only by the intracerebral route, and its intracerebral virulence has been well maintained, perhaps enhanced. Mouse brain virus introduced intraperitoneally was of rather low potency in the earliest passages, increased materially after fourteen intracerebral passages, but declined to such extent after 100 intracerebral passages that the intraperitoneal technique was unsatisfactory for immunological work. This decline in intraperitoneal virulence of high passage mouse brain virus was observed both with a line perpetuated from the original without interruption and with another line started from reconstituted dry virus.

Tests for circulating virus in mice inoculated intracerebrally with 21st passage Bunyamwera virus revealed that the agent is present in the blood at the time of onset of objective illness.

*In Monkeys.* No studies, beyond those already mentioned, have yet been made with Bunyamwera virus in monkeys. Rhesus 400 succumbed following inoculation of the mosquitoes which were the source of the virus. Rhesus 403 had a bout of fever following inoculation of serum and liver suspension from the 1st monkey, but recovered without becoming seriously ill. Rhesus 415 which received Seitz-filtered serum from the 2nd monkey, showed neither fever nor other signs, but, like rhesus 403, developed antibody as result of the inoculation.

Toxic products (other than the virus) from the large inoculum of mosquito suspension may have contributed to the death of rhesus 400. Therefore it can only be stated at this time that the virus is not invariably fatal to rhesus monkeys by subcutaneous inoculation and some monkeys receiving it may escape clinical illness altogether. During a febrile response to the virus the agent is present in the circulation. Monkeys which survive develop specific humoral antibody.

*In Rabbits.* Four rabbits were inoculated subcutaneously with large doses of Bunyamwera virus. Inoculations were done 3 times at 3 day intervals for purposes of hyperimmunization. None of the animals exhibited any clinical reaction to the injections, but all of them developed antibody against the virus.

*Other Animals.* Other animals have not yet been tested.

#### DISTRIBUTION OF THE VIRUS IN TISSUES OF MICE

When it was observed that the virulence by intraperitoneal inoculation declined with prolonged serial intracerebral passage, studies were undertaken to determine the distribution of virus in certain tissues of mice and whether that distribution was altered with passage.

One experiment was done with the 10th and one with the 103rd passage virus. Sick mice were sacrificed 2 days following intracerebral inoculation. Their tissues were removed and placed in separate sterile petri dishes—the tissues from 2 mice being used in each test. The dishes containing each tissue were weighed, and the weight of tissue was determined by weighing back the empty dishes. Ten per cent weight-to-volume suspensions were prepared with serum-saline

diluent, the trituration being facilitated by the use of finely powdered sterile Pyrex glass. In each experiment the 10 per cent suspensions were spun together in the angle centrifuge for 20 minutes at approximately 3,000 r.p.m., after which supernates were removed and dilutions of the separate tissue suspensions were prepared in serum-saline diluent. Each dilution of each tissue was tested by intracerebral inoculation of 12 mice. All mice in the 2 experiments were between 44 and 47 days old. Results of the tests are shown in table 2.

This experiment demonstrated a widespread presence of virus in the tissues, possibly accountable by the presence of virus in the circulation. In both tests, however, there was more virus in the spleen than in other tissues, brain excepted, and less in the liver.

In view of the presence of virus in the blood, the results with liver indicated the possibility that this tissue may contain something which is deleterious to the

TABLE 2

*The distribution of low and high passage Bunyamwera virus in tissues of mice inoculated intracerebrally*

TISSUE	ROUTE INOCULATED	10TH PASSAGE, TITER, 1 IN	103RD PASSAGE TITER, 1 IN
Lung.....	I-C*	500	62
Heart.....	I-C	100	0
Spleen.....	I-C	21,500	3,650
Kidney.....	I-C	460	71
Liver.....	I-C	0	trace†
Liver.....	I-P	0	
Brain.....	I-C	28,200,000	3,170,000
Brain.....	I-P	3,250	

\* I-C indicates intracerebral test and I-P indicates intraperitoneal test for virus content of tissue.

† Only one of 12 mice receiving the strongest suspension succumbed, but this animal exhibited specific signs of illness.

virus. Two experiments were done to study this possibility. In the 1st experiment 1 ml. portions of 10 per cent mouse brain virus supernate in 10 per cent serum-saline were mixed with (a) an equal portion of 10 per cent suspension of liver of normal mice, prepared in the same diluent, and (b) an equal portion of 10 per cent suspension of brains of normal mice, also prepared in the same diluent. The mixtures were incubated 2.5 hours at 37.5°C., after which serial decimal dilutions of each were made in serum-saline. Twelve mice were inoculated intracerebrally with each dilution of each mixture. The titer of virus incubated with normal mouse brain was 1 in 5,000,000, while that of virus incubated with normal liver was 1 in 12,300,000. This experiment failed to show any deleterious effect on the virus of materials extractable from liver with the serum-saline diluent.

In the 2nd experiment 0.5 ml. portions of 10 per cent virus supernate were added to (a) 4.5 ml. of physiological sodium chloride, and (b) 4.5 ml. of 1 per cent sodium tauroglycocholate (sterilized by boiling, pH 6.8). Each mixture therefore contained 1 per cent of mouse brain virus in 1 per cent serum-saline,



but mixture b contained also 0.9 of 1 per cent sodium tauroglycocholate. The mixtures were incubated for 3 hours at 37.5°C., after which serial decimal dilutions were made in 10 per cent serum-saline and tested by intracerebral inoculation into groups of 12 mice. Ten of the 12 mice receiving the mixture containing 0.9 per cent sodium tauroglycocholate were killed within a few hours (previous experience had shown that 1 per cent taurocholate is toxic by intracerebral inoculation), but all other mice receiving the dilutions of virus-tauroglycocholate mixture survived through the 12-day period of observation without becoming ill. The control, virus incubated with physiological saline, was fully potent and gave a virus titer of 1 in 21,500,000. This experiment indicated that the (impure) 0.9 per cent sodium tauroglycocholate solution had inactivated the 1 per cent virus.

#### LESIONS INDUCED BY THE VIRUS

In view of the striking symptoms induced in mice by Bunyamwera virus, the lesions caused by it are unexpectedly insignificant. The only visceral lesions encountered with any degree of frequency were in the kidneys. These consisted of marked congestion, in some instances associated with small hemorrhages in the first portion of the medulla, moderate degenerative change in the tubular epithelium and albuminous deposits in the lumina. In a few instances granular casts were present. These renal lesions were present not only in mice but in the rhesus monkey No. 400 which received the virus-containing mosquitoes.

Brains of mice infected with the virus showed marked congestion, both macroscopically and microscopically. Foci of hemorrhage were not infrequently seen, and several animals exhibited hemorrhage into the lateral ventricles. Perivascular infiltration was not a characteristic feature. Degeneration of nerve cells was present in all animals and involved especially the cells of Ammon's horn and the basal nuclei. This was characterized in some instances by vesicular change with disorganization of the chromatin pattern—apparently the earlier reaction—or by shrinking and pyknotic degeneration with fragmentation of the ganglion cells. In areas exhibiting these lesions a sparse scattering of polymorphonuclear leucocytes was sometimes present but leucocytic infiltration was often absent and never pronounced. Inclusion bodies have not been encountered.

No other noteworthy changes were observed in mice. Rhesus 400, which succumbed during infection with Bunyamwera virus, was not examined for lesions of the central nervous system as infection with a neurotropic virus was not then suspected.

#### FILTERABILITY OF THE VIRUS

One line of Bunyamwera virus was established in mice by intracerebral inoculation of a suspension of the liver of rhesus 400 after passage through a Seitz EK pad. Another line was recovered from rhesus 403 by inoculation of mice with Seitz-filtered serum, both these facts attesting to the filterability of the agent. The following experiments were done to ascertain the loss of virus occasioned by filtration through Seitz EK pads and to test its filterability through Berkefeld filters.

Two sick mice of the 14th passage group were sacrificed, and their brains were removed and ground in a mortar with 10 per cent monkey serum-saline to make a 10 per cent suspension. This was spun for 20 minutes at approximately 3,000 r.p.m. in the angle centrifuge, after which the supernate was removed and divided into 2 equal portions. One portion was passed through a Seitz EK pad under 15 pounds air pressure. Serial decimal dilutions were prepared from the unfiltered suspension and from the filtrate, and groups of 6 mice were inoculated intracerebrally with 0.03 ml. portions of each of the several dilutions of each suspension. Titers were calculated by the method of Reed and Muench (8).

TABLE 3

*Tests of filterability of Bunyamwera virus through Seitz and Berkefeld filters*

VIRUS PASSAGE USED	FILTRATE	DILUTION, LOG	FATE OF MICE		TITER, 1 IN
			Died	Lived	
14	Unfiltered	1	5	0	3,170,000
		2	6	0	
		3	6	0	
		4	6	0	
		5	6	0	
		6	6	0	
		7	0	6	
		8	0	6	
14	Seitz	1	6	0	675,000
		2	6	0	
		3	6	0	
		4	6	0	
		5	6	0	
		6	2	3	
		7	0	6	
		8	0	6	
21	Unfiltered	2	12	0	
	Berk. V	2	12	0	
	Berk. N	2	12	0	
	Berk. W	2	12	0	

Twenty-first passage mouse-brain virus was used for tests of filterability through Berkefeld filters. A 10 per cent suspension of brains of sick mice was prepared in serum-saline. This was spun for 20 minutes in the angle centrifuge at about 3,000 r.p.m.; the supernate was removed and diluted tenfold, and 10 ml. portions of this 1 per cent suspension were filtered through small Berkefeld V, N, and W filters under negative pressures not exceeding 400 mm. of mercury. Filtrates and the unfiltered suspension were tested by intracerebral inoculation into groups of 12 mice.

Results of these experiments, given in table 3, indicated that the virus passed readily through Berkefeld filters of each grade, and that it passed through the

Seitz filter without marked loss of potency. The filterability through Seitz pads was further confirmed by the experiment shown in table 1.

#### PRESERVATION OF THE AGENT

Bunyamwera virus has been successfully preserved by drying while frozen.<sup>6</sup> Considerable loss of potency was observed when the titration of the original suspension was compared with a similar test made on dried material which had been stored for 20 days in the refrigerator, perhaps owing to insufficient desiccation. However, the dried material contained viable virus in quantity after 13 months in the refrigerator, as shown by the following tabulation.

Titer of virus in fresh mouse brain.....	1 in 3,950,000
Titer of virus 20 days after being dried.....	1 in 100,000
Titer of virus 13 months after being dried.....	1 in 10,000

The virus has also been preserved by storage at 4°C. in 50 per cent glycerine. One lot of mouse brains so prepared on June 17, 1944, contained viable virus on December 2, 1944, and a protection test proved that the passage material derived therefrom was Bunyamwera virus.

#### VIRUS-NEUTRALIZATION TESTS

Soon after Bunyamwera virus was discovered methods were sought for the performance of protection tests or virus-neutralization tests. In its earlier passages the virus was of irregular and generally low virulence by intraperitoneal inoculation. After a few passages its intraperitoneal virulence became enhanced so that protection tests could be done by this route, although irregular results were not infrequently encountered in that a strong suspension of virus might cause the death of fewer mice than a weaker dilution. The experiment which follows illustrates this point and shows that satisfactory tests could be obtained despite this difficulty.

Two sick mice of the 18th passage group were sacrificed, and a 10 per cent suspension of their brains was made in serum-saline. This was spun for 20 minutes at approximately 3,000 r.p.m. in the angle centrifuge; the supernate was removed and used for the preparation of a series of decimal dilutions in serum-saline diluent.

Four series of tubes had been prepared in advance, 2 containing serum of a normal monkey and 2 containing "convalescent" serum of rhesus 415.

*Intraperitoneal Test.* To tubes containing 0.5 ml. portions of serum from a normal monkey or similar volumes of "convalescent" serum from rhesus 415 were added 0.25 ml. portions of various dilutions of virus, a given dilution being added to both sera from the same pipette, first to the normal, then to the "convalescent" serum. The tubes were shaken vigorously, immediately after which

<sup>6</sup> Equipment used for this purpose was as follows: dry ice made in a press from cylinder CO<sub>2</sub>, a high vacuum pump fitted with a MacLeod type gauge, and an all glass drying manifold of the Mudd-Flosdorf type. The dried virus was sealed in the ampoules with an acetylene flame while the vacuum pump was in operation.

inoculations of 0.06 ml. of the mixtures were made intraperitoneally into mice, groups of 6 being used for each mixture.

*Intracerebral Test.* To tubes containing 0.3 ml. portions of the same normal and "convalescent" monkey sera were added like volumes of respective virus dilutions. The tubes were shaken and the mixtures were incubated 3 hours at

TABLE 4

*Results of intracerebral and intraperitoneal neutralization tests with Bunyamwera virus*

SERUM	ROUTE INOC.	VOLUME INOC. ML.	VIRUS DILUTION, LOG*	RESULT		TITER OF VIRUS, 1 IN
				Died	Lived	
Normal rhesus monkey . . . . .	I-C	0.03	2	6	0	3,640,000
			3	6	0	
			4	6	0	
			5	6	0	
			6	4	2	
			7	1	5	
			8	0	6	
Convalescent, rhesus monkey 415 . . . . .	I-C	0.03	2	4	2	417
			3	2	4	
			4	1	5	
			5	0	6	
			6	0	6	
Normal rhesus monkey . . . . .	I-P	0.06	7	0	6	230,000
			1	6	0	
			2	5	1	
			3	6	0	
			4	4	2	
			5	3	3	
			6	4	2	
Convalescent, rhesus monkey 415 . . . . .	I-P	0.06	7	0	6	<10
			1	0	6	
			2	0	6	
			3†	0	6	

\* The actual content of virus in mixtures inoculated intracerebrally is half the indicated strength; in mixtures inoculated intraperitoneally it is one-third the indicated strength.

† 3 additional virus dilutions were tested intraperitoneally against this serum but all mice lived and the results are omitted from the table.

37.5°C., after which intracerebral inoculations were made into groups of 6 mice. Each mouse received 0.03 ml. of a serum-virus mixture. This method was the same as that used in studies on another neurotropic virus (10).

These procedures constituted intraperitoneal and intracerebral titrations of the virus in normal and convalescent monkey serum. The mice in the tests were observed for 15 days, but there were no deaths after the 10th day, nor any other signs in the surviving mice attributable to the virus. Results of the experiment are shown in table 4.

The irregular results obtained with the virus in intraperitoneal titrations are well illustrated by the data in table 4. For example, the mortality in mice receiving 1 in 100,000 virus with normal serum was lower than in those receiving the same serum mixed with 1 in 1,000,000 dilution of virus. Nevertheless, the potency of the virus by intraperitoneal inoculation was such that the neutralization of the agent by specific immune serum was easily demonstrable. Intraperitoneally the convalescent serum gave complete neutralization of the strongest dilution of virus tested, which proved to be 230,000 intraperitoneal MLD.

Intracerebrally the result was also quite convincing, although complete neutralization of the higher concentrations of virus did not take place. A  $\frac{5}{6}$  protection ratio was obtained with convalescent serum mixed with 364 MLD of virus, and all mice receiving this serum mixed with more dilute virus were protected.

The intraperitoneal test has the advantages of requiring no incubation and of giving more complete neutralization of virus, but it has the disadvantage of giving somewhat irregular mortality in the absence of specific antibody. This disadvantage might lead to unsatisfactory results in experiments in which the titer of virus is relatively low or if the serum is tested against only 1 dilution of virus.

It is also worthy of re-emphasis that very early or very late passage mouse brain virus was of insufficient intraperitoneal virulence for satisfactory protection tests by that route.

The intracerebral neutralization test as described above was employed in studies to determine whether the 4 original lines of Bunyamwera virus were similar or not. Full details of these tests need not be given here, as they add nothing to our knowledge of the essential properties of the virus, and because the volume of data accumulated was large. Briefly the experiments were as follows: Four rabbits were bled to obtain normal serum, then each was hyperimmunized with one or other of the 4 lines of virus. Intracerebral cross-neutralization tests were done with the 4 lines of virus and the 4 hyperimmune sera, using preinoculation sera as the normal control and titrating each line of virus in each of the sera. Each of the hyperimmune rabbit sera neutralized not only the homologous line of virus but also the other lines. The 4 immune sera differed in potency, and each serum had the same order of potency against every line of virus. That is, the serum which was most potent against one line of virus was also most potent against the others, and *vice versa*. This result proved the immunological identity of the 4 lines of virus.

*Differentiation from Other Neurotropic Viruses.* The intracerebral neutralization test as described above was used to determine whether Bunyamwera virus is neutralized by antisera against other neurotropic viruses. The reactions induced by the virus in experimental animals suffice to differentiate it with certainty from the yellow fever, Bwamba fever (9) and West Nile (10) viruses; nevertheless sera against these were included in the test. Results of the experiment are shown in table 5.

This experiment showed that Bunyamwera virus is not identical with the viruses of yellow fever, Bwamba fever, St. Louis or Japanese B encephalitis,

eastern or western equine encephalomyelitis, or with Semliki Forest or West Nile viruses. Neither is it identical with another undescribed virus isolated in this laboratory, now provisionally called "Lot 6 virus." Similar tests which need not be given here in detail, have shown that antisera against Rift Valley fever and others containing antibody against 6 different strains of horse-sickness virus<sup>7</sup> likewise failed to neutralize. Whether Bunyamwera virus is in any way related to these or other neurotropic viruses has not been established, but the probability seems great that it is hitherto unknown.

*Immunity Tests with Human and Animal Sera.* At the time this virus was isolated we had in hand the residues of a number of sera collected prior to the isolation of the virus from humans residing in Bwamba. The sera of 53 persons

TABLE 5

*Intracerebral neutralization tests with Bunyamwera virus and antisera against other neurotropic viruses, establishing its separate identity*

SERUM OF MONKEY IMMUNE AGAINST	PROTECTION RATIOS* IN MICE RECEIVING SERUM MIXED WITH VIRUS DILUTED 1 IN					
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>
Bwamba fever virus.....	0/6	0/6	0/6	3/5		
West Nile virus.....	0/6	0/6	0/6	3/6		
St. Louis encephalitis virus.....	0/6	0/6	0/6	1/6		
Japanese encephalitis virus.....	0/6	0/6	0/6	2/6		
Eastern equine encephalomyelitis virus.....	0/6	0/6	0/6	0/6		
Western equine encephalomyelitis virus.....	0/6	0/6	0/6	1/6		
Lot 6† virus.....	0/6	0/6	0/6	1/6		
Yellow fever and Semliki Forest virus‡.....	0/6	0/6	0/6	2/6		
Non-immune control.....	0/6	0/6	0/6	0/5	2/6	6/6
Bunyamwera virus§.....	4/6	6/6	6/6	6/6		

\* Numerator indicates the number of mice which survived, the denominator the number of those inoculated which were alive on the second day.

† "Lot 6" virus is one isolated in this laboratory, studies on which have not been published.

‡ The one animal was immune against both viruses as result of experimental procedures.

§ Rhesus 415, immune as result of a single subcutaneous injection of the virus.

residing in localities on the edge of the Semliki Forest were selected for testing, since the virus came from mosquitoes caught in that forest. Samples from 27 children and 26 adults residing in 12 forest-edge localities were chosen. The age of the youngest donor was 5 years and that of the eldest was 60. All sera were tested by the intracerebral technique. All were non-protective, indicating that infection of humans with the virus had not been common prior to August 1943, when the samples of blood were taken. This result did not prove, however, that humans were unaffected by the virus at the time it was known to be active, in September 1943. The results which follow show that a significant number of persons were immune 11 months after the isolation of the virus.

<sup>7</sup> The horse-sickness sera were generously supplied by Dr. S. E. Piercy of the Veterinary Laboratory, Kabete, Kenya.

Sera of 298 residents of Bwamba County, taken in August 1944, were tested against the virus by the intracerebral method, each serum originally being tested against 2 decimal dilutions of virus. The extremes of virus titer in the 9 test runs were such that the test doses of virus ranged between 23 and 2,300 intracerebral MLD. Many of the sera gave clear-cut protective or negative results in the first tests, but others gave results less easily interpreted. Some of the latter sera gave partial but incomplete protection; others gave no protection, as evidenced by reduced mortality, but did permit increased survival time in mice. Therefore a study was made to determine the limits of sensitivity of the intracerebral test and to learn what was the minimum variation from normal which could be regarded with certainty as specific neutralization of virus.

Several of the sera were tested a second time against 3 dilutions of virus with 6 mice for each serum-virus mixture, but results with a few were still not decisive. Accordingly pools were made of 2 to 5 specimens of clearly protective sera, clearly non-protective specimens and others which we shall here designate as doubtful. All sera included in any one of the "doubtful" pools had given very similar or identical results in the first or second tests, but the different pools were made up of sera which had given very different reactions, some being regarded as probably negative and others as almost certainly protective. (Sera were pooled only in order to have sufficient quantities for the tests to be carried out.) Each serum pool was filtered through a Seitz EK pad, and 1 ml. portions were placed in each of 5 tubes. Mouse brain virus suspension was prepared in 10 per cent serum-saline and cleared by centrifugation, after which dilutions were prepared to 1 in  $10^{-5}$  in the same diluent. One milliliter portions of 5 dilutions of virus were added to the tubes of each serum pool and the mixtures were incubated for 2.75 hours at  $37.5^{\circ}\text{C}$ . air temperature. Groups of 12 mice of identical age—42 days—were then inoculated intracerebrally with each serum-virus mixture, each mouse receiving 0.03 ml. Known non-immune and immune monkey sera and the serum of an immune rabbit were used similarly as controls. Preparation of the serum-virus mixtures required about the same amount of time as the inoculations. Mixtures first prepared were first inoculated in order to minimize variations which might be occasioned by differences in contact time. All inoculations were done by 1 person. Results of the test, including the time of death in mice, are shown in table 6.

The data on survival time in the foregoing experiment were submitted to statistical analysis and it was found that serum pool A was not significantly different from normal serum. In the preliminary tests the mice receiving sera comprising this pool lived longer than control mice, but all or nearly all died. It is probable that the result in the final test approaches the maximum deviation to be expected with non-immune sera.

Serum pools B, C, D, and E gave results which were significantly different from those with normal serum and are therefore to be considered as immune sera. It is noteworthy that the potency of each was less than that of the known immune human serum. The latter represents the most potent human serum we have encountered, while pool B probably represents an approach to the minimum of neutralization which can be regarded as specific.

TABLE 6

*Intracerebral protection test to study the sensitivity of the method*

SERUM	VIRUS DILUTION, LOG	DEATHS OF MICE, DAYS										FATE OF MICE		TITER OF VIRUS, 1 IN
		1	2	3	4	5	6	7	8	9	10	Died	Lived	
Normal rhesus 557.....	4				8	4						12	0	2,780,000
	5				9	2	1					12	0	
	6				1	3	2		3			9	3	
	7					1	1					2	10	
	8											0	12	
Normal human pool....	4			4	7	1						12	0	2,820,000
	5				5	5	1	1				12	0	
	6				1	4	3	1	1			10	2	
	7				1							1	11	
	8											0	12	
Human pool A.....	4				11	1						12	0	395,000
	5				3	7	2					12	0	
	6						1			1		2	10	
	7											0	12	
	8											0	12	
Human pool B.....	4				1	3	3	1			1	9	3	69,000†
	5					1	3	1	1			6	6	
	6						1					1	11	
	7											0	12	
	8											0	12	
Human pool C.....	4				1	5	4		1		1	12	0	64,200†
	5					1	1		1		1	4	8	
	6						1					1	11	
	7											0	12	
	8											0	12	
Human pool D.....	4				2	4	1	2	2			11	1	28,200†
	5											0	12	
	6											0	12	
	7							1				1	11	
	8		1*									0	11	
Human pool E.....	3				6	6						12	0	57,500†
	4				3	2	6					11	1	
	5					1	2			1		4	8	
	6										1	1	11	
	7											0	12	
Human known immune.	3					1	2	1	1	1		6	6	2,900
	4					2	1	1				4	8	
	5					1						1	11	
	6					1						1	11	
	7											0	12	
Immune rhesus 415.....	3							1	1			2	10	<1,000
	4											0	12	
	5		1*	1*		1						1	9	
Immune rabbit 21.....	2				5	5	1				1	12	0	4,170
	3				1	3	2	2		2	1	11	1	
	4					1	1				1	3	9	
	5											0	12	
	6										1	1	11	

\* Non-specific deaths.

† Unknown sera which gave results significantly different from non-immune sera.



Serum pool B (table 6) was the weakest one which gave significant neutralization in this quantitative test. The sera included in that pool each neutralized just 1.33 decimal dilutions of virus (20 MLD) in the preliminary experiments employing 3 dilutions of virus. Neutralization of 20 MLD of virus was therefore adopted as the minimum, and any serum which neutralized less than 20 MLD was regarded as negative. Only 2 sera (those included in pool B) were encountered which were as weakly protective as this. Most potent among the protective sera encountered was one which gave complete protection against 610 intracerebral MLD of virus and the "known human immune pool" (table 6) which neutralized nearly 1,000 MLD.

TABLE 7

*Results of survey of immunity against Bunyamwera virus in Bwamba County, Uganda, one year after isolation of the virus*

AREA SAMPLED	AGE GROUP	NO. TESTED	PROTECTIVE		AGE OF YOUNGEST IMMUNE
			Number	Per cent	
					<i>years</i>
Bubandi.....	Children*	30	3	10.0	8
	Adults	29	4	13.8	
Busaro.....	Children	35	2	5.7	7
	Adults	35	4	11.4	
Buhundu.....	Children	30	0	0	
	Adults	29	2	6.9	30
Hakitengya.....	Children	35	1	2.8	10
	Adults	35	2	5.7	
Rwebisengo.....	Children	20	4	20.0	10
	Adults	20	6	30.0	
Total.....	Children	150	10	6.7	
	Adults	148	18	12.2	

\* Persons under 15 years of age were recorded as children.

Of the 298 sera collected from Bwamba residents in 1944, 28 were found to be protective, as shown in table 7. The negative results in the 1943 tests followed by the finding of immune individuals in 1944 did not prove that any one of these persons was immunized in that interval, as none of the immune donors were included in the previous year's sample. The results do seem to indicate an increase in the incidence of immunity during that year, and point to the Rwebisengo area as the most heavily infected region.

The five areas (table 7) from which samples of human sera were tested against Bunyamwera virus differ greatly from each other. Bubandi and Busaro, adjacent regions in the southern part of Bwamba are most similar. These areas are characterized by undulating grassland and cultivated tracts. The altitude is about 3,000 feet above sea-level. There is relatively little forest, and that which is present is in small gallery extensions along the streams. The population density is high.

The Buhundu area is mountain grassland interspersed with cultivated plots, interrupted along the streams by small gallery forests. The area is on the lower slopes of the Ruwenzori Mountains, at an altitude varying between 4,000 and 7,000 feet. The population is scanty in comparison with that of the Bubandi-Busaro or the Hakitengya area.

The latter region is situated at about 3,000 feet and is characterized by mixed cultivation and bush country, broken frequently by deep ravines, the slopes of which are covered with dense forest.

The Rwebisengo area is on the unforested plains of the Semliki River at an altitude of about 2,500 feet. There is relatively little cultivation, but much grazing. The population is scanty. The fact that this was the area in which the highest incidence of immunity against the virus was found, may give some clue as to possible insect vectors (if, indeed, it is insect borne), as it is the only one of the areas which has no forest of consequence.

Thirty-one sera from persons who had recently suffered attacks of illness of varied nature were also tested against the virus. Seven of these were from persons who had poliomyelitis, 4 from persons whose illness did not suggest infection with a neurotropic virus and the remainder from persons with suspected virus encephalitis. All were non-protective except one of the latter. This serum<sup>8</sup> gave complete protection against 230 mouse intracerebral MLD of virus, and the donor is therefore presumed to have suffered infection with this virus. It cannot be stated, of course, that the suspect illness was due to this virus, but the protective result with convalescent serum is evidence that it may have been.

Sera of 42 monkeys shot or captured in Bwamba were tested against the virus. Each was negative, with 1 exception—a black-and-white colobus monkey which was 1 of 2 shot in the catching area during the time of capture of the mosquitoes from which the virus was isolated. All the other monkey sera were non-protective, but it is noteworthy that the great majority of the specimens were collected in uninhabited forest several miles from Bunyamwera III.

#### DISCUSSION

Bunyamwera virus does not, so far as we know, attack selectively the cellular elements of nervous tissue. In experimental animals, however, it elicits neurological reactions and exerts its principal effects on the nervous system, regardless of the route by which it is introduced. Therefore we have referred to it as a neurotropic virus, without intending to imply a particular affinity for nerve cells.

From what is known, one could not predict the effects which the virus might have in man. The 1 human case of obscure illness in which humoral immunity to Bunyamwera virus was subsequently found cannot be regarded with certainty as having been caused by that agent. The information at hand indicates only that the individual, at some time previous to being bled, had an experience with this virus. It might even be that, if the virus does cause clinical illness in man, such illness would not be characterized by marked neurological signs. This is

<sup>8</sup> For the 2 samples of blood from this patient we are indebted to Dr. J. C. St. George Earl, Senior Medical Officer in Charge, Zanzibar, and Dr. J. D. Robertson, Pathologist, Zanzibar.

the case in Bwamba fever (9), a benign self-limited disease not characterized by marked neurological symptoms, but caused by a virus which, in mice, induces more profound lesions in the central nervous system than the agent with which we are here concerned. It is possible also that the host of preference for Bunyamwera virus is not man but some wild animal. Even if that is the case it could still be of medical importance, as is the virus of yellow fever, which attacks both the animals of the jungle and man. Further investigation may throw light on these points.

In recent years a considerable number of previously unknown filterable viruses have been discovered, many of them being neurotropic in action. These include the viruses of eastern (11), western (12) and Venezuelan (13) equine encephalomyelitis; lymphocytic choriomeningitis (14); St. Louis (15), Japanese B (16) and Russian spring-summer (17) encephalitis; and louping ill (18), with which the Russian virus may be identical (19). Also, in this laboratory, and quite incidental to our main problem (yellow fever), we have encountered 4 hitherto unknown viruses (5, 9, 10, and present communication) and have found humoral immunity to each to occur in man. Some of these agents have been isolated from man or animals, others from insects and a few from both. Some have been discovered during the course of investigations of specific diseases in man or animals, while others were encountered accidentally, so to speak. Some are related antigenically, others are not known to be. The significance of at least 3 of the agents in human and/or veterinary medicine is not yet known (5, 10, and present communication). All of these facts indicate that a concerted attack on the problem of virus etiology of disease (including pyrexias of unknown origin) might not only increase our knowledge of the known viruses and their properties but also uncover further viruses at present unknown as the causative agents of disease.

#### SUMMARY

1. A filterable virus, believed to be hitherto unknown, has been isolated from *Aedes* mosquitoes caught in uninhabited forest in western Uganda. It has been provisionally named Bunyamwera virus in respect of the locality in which it was encountered.

2. The agent is pathogenic for white mice and exerts its principal pathogenic effects on the nervous system regardless of the route by which it is introduced.

3. The effects of the virus in rhesus monkeys and rabbits are discussed.

4. The agent elicits the formation of virus-neutralizing antibodies in animals which survive inoculation. The presence of these antibodies may be ascertained by intracerebral or intraperitoneal tests.

5. Bunyamwera virus is immunologically different from the viruses of yellow fever, Bwamba fever, St. Louis and Japanese B encephalitis, Rift Valley fever, eastern and western equine encephalomyelitis and horse-sickness, from West Nile and Semliki Forest viruses and from another neurotropic virus isolated here but not yet reported.

6. Neutralizing antibody against the virus has been found in a forest monkey, in a human who suffered a recent attack of febrile illness characterized by marked neurological signs and in a number of persons sampled at random.

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# MEDICAL SHOCK IN THE PATHOGENESIS OF ALGID MALARIA<sup>1</sup>

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This paper attempts to show that the algid form of malaria is due to the development of medical shock. It would be well to start with a discussion of these terms.

Craig (1) stated, in describing algid malaria "The characteristic condition is one of extreme collapse attended by profuse perspiration, the temperature at the same time being more or less elevated, although in some cases the temperature is sub-normal. Patients suffering from this form of malaria present a characteristic countenance, the cheeks being drawn and pinched, the eyes sunken, the nostrils dilated and the skin bedewed with perspiration. The entire body is cold, the skin cyanotic and bathed with a cold sweat. The lips and finger-nails are intensely cyanotic. The tongue is tremulous, dry and coated with a dirty white fur. The pulse is rapid, thready, and easily compressible and generally more or less intermittent; the heart sounds are muffled and the second sound almost inaudible, and as death approaches the pulse becomes imperceptible; the respirations are irregular, superficial in character and labored; the muscular weakness is extreme, while the mental condition of the patient is one of apathy to his surroundings and indifference as to his condition. The symptoms rarely last over a few hours, death generally resulting."

Manson-Bahr (2) divided "pernicious attacks" of malaria into cerebral and algid forms. "The algid forms of pernicious attack, as indicated by the name, are characterized by collapse, extreme coldness of the surface of the body and a tendency to fatal syncope." His algid forms included gastric, choleraic, dysenteric, hemorrhagic, and syncopal types. Strong's (3) discussion was along almost identical lines.

Bercovitz (4) wrote, "In one of the most serious types of malaria the symptoms usually develop after one or more ordinary malarial paroxysms. There is usually a condition of extreme collapse attended by profuse perspiration, the temperature at the same time being more or less elevated, although it may be subnormal. The cheeks are drawn and pinched, the eyes sunken, the nostrils dilated, the skin covered with perspiration and cyanotic. The entire body is cold, the fingernails and toenails cyanotic. The pulse is rapid, thready, intermittent or irregular, and the heart sounds muffled. In some instances patients with pernicious falciparum malaria may have symptoms resembling Asiatic cholera."

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Nocht and Mayer (5) included the following form in the pernicious types: "Algid and cardiac form (reminiscent of the algid stage in cholera): rapid loss of strength, extreme cardiac weakness, Hippocratic facies, threatening collapse, intense perspiration, and cool skin. Whilst the temperature in the arm pit is comparatively low that in the anus is high."

These descriptions by present day authorities provide a picture of the clinical syndrome of algid malaria.

The clinical signs and symptoms of surgical or traumatic shock are well known. In recent years the importance of hemoconcentration in shock, as determined by erythrocyte counts, hematocrit studies, and specific gravity determinations, has received wide recognition. According to Atchley (6) "Medical shock is a term denoting for the internist the complication which, when it occurs in surgery, is called surgical shock. Shock, whenever it occurs, is a serious and often fatal condition characterized by vasomotor collapse. . . ." He considered diabetic acidosis, pneumonia, typhoid, and influenza to be conditions which would cause medical shock. Of even more interest was the emphasis by Atchley and Loeb (7) on the role which shock, accompanied by anhydremia and hypochloremia, plays in Asiatic cholera. They indicated that infusions of salt solution and even transfusions would counteract the physiologic imbalance produced by the disease.

Fishberg (8) stated that "Except in rheumatic fever and probably diphtheria, circulatory failure in the acute infectious diseases is far more often of peripheral than of cardiac origin. The clinical picture is fundamentally the same as that of traumatic shock, and differs only in those details which are due to the terrain of the infectious disease in which it occurs. . . ."

Moon (9, 10, 11) recently summarized the acute infections which cause medical shock. In addition to those diseases which have already been discussed, he specifically mentioned gas gangrene, diphtheria, typhus, yellow fever, puerperal sepsis, scarlet fever, erysipelas, and plague. All authors emphasize the importance of intravenous replacement therapy in restoring the deficient circulating blood volume. In most cases this reduction of blood volume is manifested by hemoconcentration due to plasma loss through the damaged capillary bed.

Although many authors have described the algid symptoms of malaria, only a few have employed the term or concept of shock.

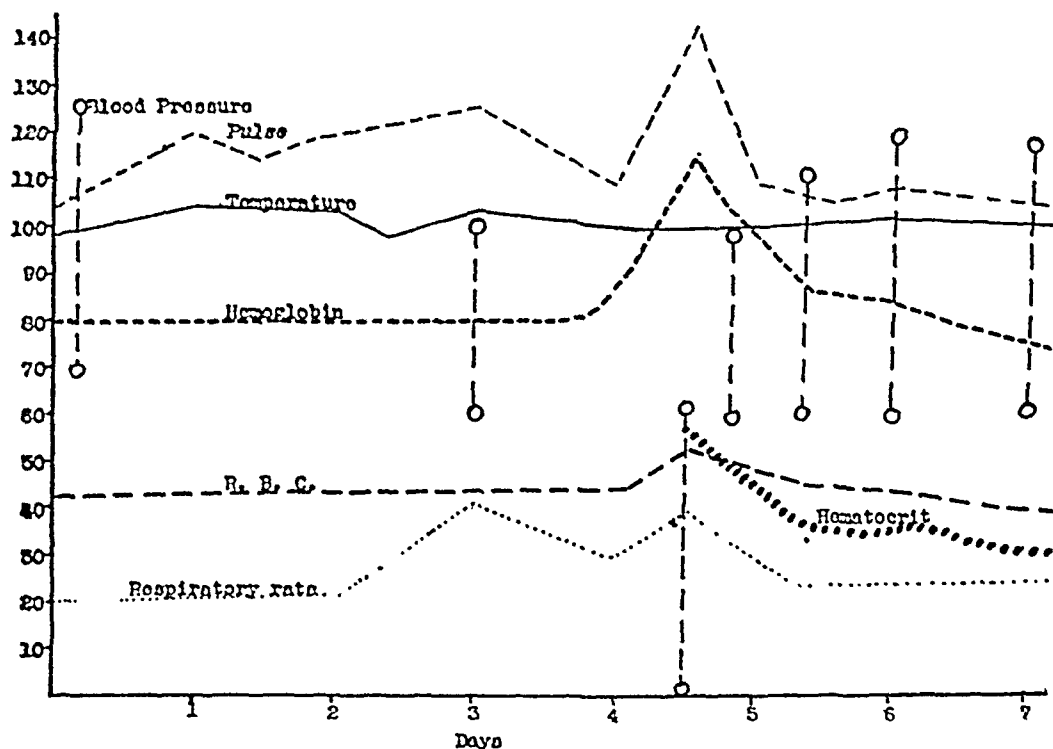
The earliest record that we have been able to find of the direct comparison of algid malaria with shock was an article on algid malaria by Gage (12), published in 1926. He stated "The patients present a picture almost similar to that of acute shock, so frequently seen in severe trauma, and, happily less often, in surgery." His description of patients with algid malaria may be abbreviated as follows: "Such patients are unable to stand. . . and they are cold and frequently covered with 'clammy' perspiration; pulse is very rapid, thready, and at times not palpable at the wrist. . . the blood pressure is markedly decreased. . . there is great effort involved in talking. . . the eyes appear sunken and the expression anxious. . . vomiting. . . diarrhea is sometimes present. . . The skin is pale, a symptom in sharp contrast to the color of the skin in some of the other forms of

malaria more chronic in character. . . .The temperature by mouth is generally subnormal. . . ." He presented five case histories and outlined a course of treatment which included general supportive measures and intramuscular quinine, but no intravenous therapy.

Cannon (13) wrote: "One feature of pernicious malaria which is noteworthy is the vascular injury as revealed by generalized fatty degeneration, hemorrhages into the brain, purpura, etc. Such a condition should certainly predispose to loss of fluid elements of the blood similar to that in shock, as it does particularly in the algid forms of pernicious malaria. There is also evidence of hemoconcentration in pernicious malaria at times, which might also lead, as afore mentioned, to an increased tendency to blockage of capillaries." Rigdon (14) reported the case of a 7 year old girl who died of acute estivo-autumnal malaria with the clinical and postmortem findings of shock. Rigdon and Stratman-Thomas (15) described pathologic changes simulating those of shock in monkeys who died with severe *Plasmodium knowlesi* infections although they did not employ that term. Kean and Smith (16), in a report based on 100 autopsies on patients who had died of estivo-autumnal malaria, believed that 20 per cent died of shock. Elevated plasma potassium levels have been recorded in malaria infections—in man by Zwemer, Sims and Coggeshall (17), and in canaries by Velick and Scudder (18). The significance of plasma potassium levels in shock is still a moot question; it has been suggested that the high plasma potassium in malaria may be secondary to hemolysis of the erythrocytes which have a high potassium content.

*Case 1.* A 39 year old white male who had no history of previous malaria or other major illnesses, was admitted to the hospital on November 28, 1943. On the day before admission he had experienced chilly sensations, malaise, upper abdominal distress, and he felt feverish. At the time of admission he was "quite ill," flushed, and perspiring moderately. Except for slight epigastric tenderness, physical examination was negative. His temperature was 99.4°F., his pulse rate 105 per minute, and his respiratory rate 20 per minute. His blood pressure was 130 mm. of mercury systolic and 70 mm. diastolic. Hemoglobin was 80 per cent (Tallqvist), erythrocytes were 4,350,000 per cu. mm. of blood, leukocytes 5,235 per cu. mm. of blood, with 61 per cent neutrophils, 37 per cent lymphocytes, and 2 per cent eosinophiles. Ring forms of estivo-autumnal malaria were found in smears of the peripheral blood and the patient was given 0.2 gram of atabrine by mouth shortly after admission and 0.1 gram four hours later. On the second and third days he received 0.1 gram of atabrine every eight hours. The temperature rose to 105°F. on the day after admission and the number of parasites increased progressively. On the third hospital day 2 per cent of the erythrocytes contained ring forms; on the morning of the fourth day 3 per cent were parasitized. At this time cyanosis and capillary stasis of the skin were noted. The blood pressure decreased to 100 mm. of mercury systolic and 60 mm. diastolic, and the pulse rate rose to 130 per minute. Intravenous fluids were given and 0.1 gram of atabrine was administered intramuscularly and 0.1 gram orally.

About noon of the fourth hospital day the patient went into profound shock, and also showed the clinical manifestations of algid malaria. The blood pressure fell as low as 64 mm. of mercury systolic and 0 diastolic, the radial pulse was almost imperceptible, the heart rate was about 155 per minute, the respiratory rate was 40 per minute, and the temperature remained about 101°F. orally. The hematocrit was 57, the hemoglobin 114 per cent (Sahli), and there were 5,080,000 erythrocytes per cu. mm. of blood. The sedimentation rate was 5 mm. in one hour (Westergren). Five per cent of the erythrocytes were parasitized at this time. Following the first appearance of a falling blood pressure



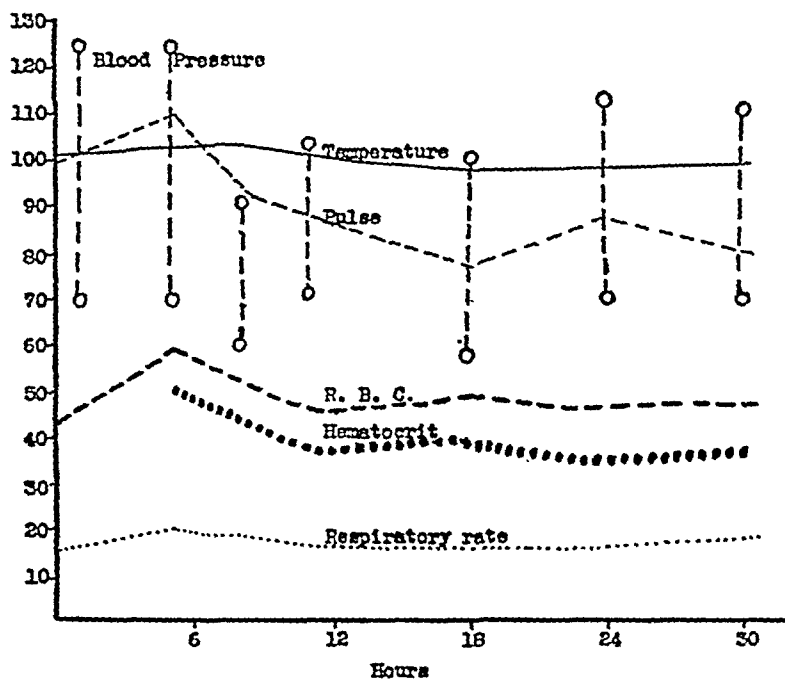
CASE 1

plasma infusions were started, together with the constant administration of intravenous fluids and oxygen. At noon of the fourth day the patient was given 0.5 gram of quinine dihydrochloride intravenously and this was repeated at 9:00 p.m., and 7:00 a.m. the next day. In addition, 1.0 gram of quinine sulfate was given orally at 3:00 p.m. and 8:00 p.m. of the fourth day and at 3:00 a.m. of the fifth day. Two grams of quinine were given orally during the remainder of the fifth day.

A definite clinical response was observed with each of the three 300 cc. infusions of plasma. (Three cubic centimeters of "Eschatin" were given simultaneously.) With each unit of plasma there was subjective improvement, the patient being relieved of his apprehensive, semistuporous mental state. There were also objective changes such as a blood pressure rise from 64 mm. of mercury systolic and 0 mm. diastolic to 100 mm. systolic and 60 mm. diastolic.



Fifteen hours after the onset of the shock syndrome the patient showed definite clinical improvement. His blood pressure was 112 mm. of mercury systolic and 60 mm. diastolic, the pulse rate was 108 per minute, and the respiratory rate 24 per minute. The erythrocytes still showed 5 per cent parasitization on the fifth hospital day, the hemoglobin being 88 per cent (Sahli), erythrocytes 4,650,000 per cu. mm., and the hematocrit 37. The urine contained granular and hyaline casts and 1 plus albumin. The nonprotein nitrogen was 68.3 mg. per 100 cc. of blood, the creatinine 3.7 mg. per 100 cc. of blood, and the potassium 14.0 mg. per 100 cc. of serum. There was gradual defervescence during the following nine days while the patient continued to receive 2.0 grams of quinine by



CASE 2

mouth each day. The number of parasites in the blood gradually decreased and on the eighth day they disappeared. At this time the hematocrit was 30 and the icterus index was 30. Two weeks after admission the sedimentation rate was 124 mm. in one hour (Westergren). All blood findings gradually returned to normal. Electrocardiograms were negative throughout except for slight evidence of myocardial damage. On the seventh hospital day he became irrational, incoherent, and quarrelsome, showing paranoid ideas. The psychotic manifestations disappeared after two days and there were no residual changes. Three months later this patient was readmitted to the hospital with a mild hepatitis. His blood pressure was 128 mm. of mercury systolic and 76 mm. diastolic. His blood findings were negative except for the changes associated with the jaundice.

*Case 2.* A 32 year old white male was admitted to the hospital on August 27, 1942. In 1934 he had malaria in Haiti with an uncomplicated response to

treatment. Three days before admission he developed headache and backache, and subsequently he experienced chills and fever. For two days he had vomited occasionally and had mild diarrhea. On admission his temperature was 100.8°F., his pulse rate 100 per minute, his blood pressure 130 mm. of mercury systolic and 70 diastolic, and his respiratory rate was 16 per minute. Physical examination was essentially negative. Examination of his blood revealed hemoglobin 75 per cent (Tallqvist), erythrocytes 4,230,000 per cu. mm., leukocytes 4,100 per cu. mm., with a differential count of 65 per cent neutrophils and 35 per cent lymphocytes. Approximately 1 per cent of the erythrocytes were infected with *Plasmodium falciparum*. He was given 0.75 grams of quinine by mouth two hours after admission. Because of vomiting 0.2 gram of atabrine was injected intramuscularly two hours later. Six hours after admission the erythrocyte count had risen to 5,650,000 per cu. mm. and his hematocrit was 47. At this time the blood pressure was still 130 mm. of mercury systolic and 70 diastolic, the temperature was 103°F., the pulse rate 110 per minute, and the respiratory rate 20 per minute. Two thousand cubic centimeters of fluids with 1 gram of quinine dihydrochloride were administered intravenously; 0.5 cc. of epinephrine was injected subcutaneously. In spite of this therapy the temperature rose to 103.8°F., the patient became increasingly lethargic, his skin was cold and moist, and eight hours after admission the blood pressure had fallen to 90 mm. of mercury systolic and 60 diastolic. Intravenous fluids and the usual supportive measures were continued and he was given 1 gram of quinine and 0.1 gram of atabrine by mouth. Nine hours after admission he began to show clinical improvement. Eleven hours after admission the temperature was 101.6°F., the pulse rate 88 per minute, the respiratory rate 16 per minute, and the blood pressure 104 mm. of mercury systolic and 72 diastolic. The hematocrit at this time had fallen to 36 and the red blood cell count to 4,590,000 per cu. mm. Thirteen hours after admission he was given 1 gram of quinine and 0.1 gram atabrine by mouth. Thereafter, atabrine was discontinued and the patient received 2 grams of quinine orally in divided doses for six days. He had an uneventful convalescence, with the hematocrit and erythrocyte count remaining essentially unchanged.

Case 3. The patient was a 37 year old white soldier who had no history of previous malaria and had not received antimalarial drugs "prophylactically." His illness started with a light chill and a dizzy sensation on May 21, 1944. Nine days later he finally reported to his station hospital semidelirious and apparently dehydrated from vomiting. *Plasmodium falciparum* was found in the blood smears and he was transferred to another hospital because of the severity of his illness. On admission he was mentally confused and appeared dehydrated, although his skin was damp and clammy. Physical examination revealed a palpable, tender spleen and tenderness in the region of the liver. His blood pressure was 86 mm. of mercury systolic and 56 mm. diastolic, the rectal temperature was 99°F., the pulse rate was 92 per minute, and the respiratory rate was 30 per minute. Examination of the blood smear on admission showed 12 per cent parasitization of the erythrocytes and a few crescents. His hemoglobin was 77 per cent (Hellige), erythrocytes 3,440,000 per cu. mm., and

leukocytes 9,250 per cu. mm. The urine contained 1 plus albumin and granular casts. He was immediately given 0.2 grams of atabrine intramuscularly and 0.5 grams of quinine in 1,000 cc. of 5 per cent glucose intravenously. Two hours after admission his blood pressure had dropped to 70 mm. of mercury systolic and 50 mm. diastolic, the rectal temperature was 99.2°F., the pulse rate was 120 per minute, its quality weak and thready, and the respiratory rate was 30 per minute. Three hours after admission an infusion of 250 cc. of plasma was given, but the patient remained in shock; he was cyanotic, restless, had hiccups, and the skin remained cool and moist. Seven hours after admission the blood pressure was 66 mm. of mercury systolic and 40 mm. diastolic. Fluids were administered intravenously with an additional 0.5 grams of quinine dihydrochloride. He also received 0.5 cc. of epinephrine intramuscularly, 2 cc. of "Eschatin" intravenously, and oxygen therapy was started. Twelve hours after admission he seemed to be recovering from shock; his blood pressure was 90 mm. of mercury systolic and 60 mm. diastolic, his pulse rate was 88 per minute and of good quality, his respirations were 24 per minute, and his oral temperature was 102.6°F. During the first twenty-four hours he received a total of 3 grams of quinine and 0.4 grams of atabrine. On the second hospital day examination of the blood revealed: hemoglobin 48 per cent (Hellige), erythrocytes 2,050,000 per cu. mm., leukocytes 7,100 per cu. mm., with 94 per cent neutrophils, 1 per cent myelocytes, and 5 per cent lymphocytes; platelets 60,000 per cu. mm., 1 per cent parasitization of erythrocytes, sedimentation rate 40 mm. in one hour (Westergren), and hematocrit 32. There were no purpuric or hemorrhagic manifestations. He was given a transfusion of 500 cc. of whole blood and the intensive antimalarial therapy was continued. On the third hospital day he was much improved and could retain the usual oral quinine medication. His blood findings gradually returned to normal; crescents were found until the seventh hospital day. He regained the ten pounds that he had lost and was discharged two months after admission.

*Case 4.*<sup>3</sup> A 34 year old male was admitted to the hospital on May 29, 1942. He had never had malaria or other major illnesses. Three days before admission he developed chills, fever, headache, backache, generalized malaise, and moderate diarrhea. He appeared tired and weak on admission. His temperature was 101°F., pulse rate 82 per minute, respirations 20 per minute, and blood pressure 95 mm. of mercury systolic and 65 mm. diastolic. Except for moderate injection of the throat, dental caries, and a barely palpable spleen, the physical examination was negative. Examination of the blood showed moderate infection with *Plasmodium falciparum* and a leukocyte count of 4,200 per cu. mm. Twenty minutes after admission 2 grams of quinine sulfate were administered orally. Five hours after admission his condition was unchanged except that his temperature had risen to 102.4°F. Six and one-half hours after admission he was found in shock. The skin was cold and clammy, the pulse imperceptible, the temperature 95°F., and the blood pressure was 60 mm. of mercury systolic and 40 mm. diastolic. He had a profuse watery bowel movement. Shock

<sup>3</sup> This case was included in a previous report (15).

treatment was started immediately. His feet were elevated, heat was applied, 0.5 cc. of epinephrine was injected subcutaneously, and 1,000 cc. of 5 per cent glucose in saline followed by 250 cc. of plasma were given intravenously. Eight and one-half hours after admission the patient's condition was much improved; his pulse rate was 72 per minute and of good quality, his temperature was 96.6°F., and his blood pressure was 86 mm. of mercury systolic and 56 mm. diastolic. Eleven hours after admission his blood pressure was 100 mm. of mercury systolic and 60 mm. diastolic. Combined treatment with quinine and atabrine was continued with no further complications.

*Case 5.* A 47 year old white female was admitted to the hospital on June 2, 1942. She had never had malaria or other major illnesses. Two days before admission she developed fever, headache, malaise, and subsequently she had chills, nausea, and vomiting. Physical examination was essentially negative except that the temperature was 103°F. and the pulse rate was 120 per minute. Examination of blood showed moderate infection with *Plasmodium falciparum*, leukocytes were 6,250 per cu. mm., erythrocytes 4,020,000 per cu. mm., and hemoglobin was 75 per cent (Tallqvist). She was promptly started on oral atabrine therapy, receiving 0.1 grams three times daily. Her temperature dropped to 98°F., but sixteen hours after admission it again rose to 101.5°F. and the blood still showed moderate parasitization. She was given an additional 0.2 grams of atabrine intramuscularly. Twenty hours after admission she suddenly went into a "moderate shock state," according to the note of the attending physician. She was "nauseated, vomited, cyanotic, with a thin, thready, barely perceptible pulse." The attending physician stated that the blood pressure was significantly lowered, but did not record the exact level. Shock therapy was immediately instituted, and consisted of elevation of the feet, heat, 0.5 cc. of epinephrine subcutaneously, and 1100 cc. of 10 per cent glucose in saline intravenously. She made a satisfactory response and all the clinical manifestations of shock disappeared. Four hours later the temperature was 103°F., the pulse rate was 112 per minute, and the respiratory rate was 28 per minute. The following day, although subjectively she was much improved, the blood was still heavily parasitized. Intravenous and oral quinine therapy was instituted with good response except for the development of transient deafness and vertigo.

*Case 6.* The patient was a 25 year old, white soldier who was admitted to the hospital July 31, 1942. He had not received "prophylactic" quinine and had never had malaria. Ten days before admission he began to have fever, malaise, and muscular aches. On each of the three days before admission he had a chill. Because he was scheduled to return to the United States on furlough, he failed to report his illness until the day of admission when, following another severe chill, he was found to have a temperature of 105°F. He was acutely ill and to the clinician he appeared to be "in moderate shock." His blood pressure was 100 mm. of mercury systolic and 20 diastolic. Physical examination was essentially negative except for a tender spleen palpable 3 cm. below the costal margin and a blowing systolic murmur in the aortic area. His skin was hot and

clammy, temperature 105°F., pulse rate 120 per minute, and respiratory rate 28 per minute. There were 4,070,000 erythrocytes per cu. mm. of blood, 5,000 leukocytes per cu. mm. of blood, and the hemoglobin was 80 per cent (Tallqvist). The hematocrit was 37.5. Two per cent of the erythrocytes were infected with *Plasmodium falciparum*. He was immediately started on oral atabrine in large doses. Two hours after admission he still appeared to be in incipient shock; the blood pressure was 90 mm. of mercury systolic and 10 diastolic. Intramuscular atabrine was administered and a plasma infusion was prepared. He then started to improve so the plasma was not given. Seven hours after admission his blood pressure was 110 mm. of mercury systolic and 50 diastolic. From that time he continued to respond normally and the aortic murmur disappeared. On the second, fourth, and fifth days in the hospital his hematocrit ranged from 25 to 28 and his red blood cell count was 3,200,000 per cu. mm. Malaria parasites were not found in the smears after the second day.

#### DISCUSSION

These cases of algid malaria showed the clinical findings of medical shock including lowered blood pressure and other signs of vasomotor collapse. Hemoconcentration, evidenced by an increase in the hematocrit, red blood cell count and hemoglobin, was demonstrated in the 3 cases in which adequate studies were made. The final evidence for shock in these cases was the response to replacement therapy. Plasma was used in 3 cases, whole blood transfusion in 1, oxygen in 2, "Eschatin" in 2, intravenous fluids in 5, and epinephrine in 5. Once the shock syndrome develops appropriate supportive measures must be taken at once but specific therapy of the malaria infection should be vigorously continued.

The essential feature in shock is the disparity between the circulating blood volume and the size of the vascular bed. This may be produced by factors which have been classified by Blalock (19) and Dunphy (20) as neurogenic, vasogenic, or hematogenic. The neurogenic factor (associated with "primary" shock) and the vasogenic factor, produced by histamine, poisons, anaphylactic agents, etc., act by dilating capillaries, thus increasing the peripheral vascular bed. The more common hematogenic type is characterized by a decrease in the circulating blood volume due to loss of water, plasma, or whole blood.

Blalock (19) stated "little is known about the mechanism of the circulatory failure that occurs so frequently in typhoid fever, pneumonia, and other severe infections." It is thought that one of the three mechanisms usually initiates the shock syndrome and as the disease progresses the others become active. Aub and his coworkers (21) have shown that experimental traumatic shock in dogs is due, not only to local plasma loss caused by the trauma, but also to an enzymic bacterial "toxic factor" which is produced by *Clostridia* frequently found in the wounds. In medical shock occurring terminally in various acute infections, Stead (22), however, was unable to find any of these specific mechanisms to be primarily responsible; there was no evidence of hemoconcentration, the plasma volume was normal, and plasma and whole blood transfusions failed to improve the circulation.

In malaria, the significance of the "vasogenic" and "neurogenic" mechanisms is difficult to determine from the evidence now available, although it is possible that dilatation of the capillary bed may be caused by hemolyzed red blood cell constituents or by proteins of parasitic origin. In our cases there was more evidence for "hematogenic" shock than either of the other types. Depletion of the circulating blood volume in malaria may be produced in three ways.

1. It may be caused by the loss of water and electrolytes due to vomiting, diarrhea, and diaphoresis.

2. The hemolysis of red blood cells may be equivalent to the loss of whole blood through hemorrhage. Rigdon (13) postulated that shock in malaria may be due to anoxemia of the capillary bed caused by "rapid destruction of red cells" and "high degree of parasitization." In our cases the degree of parasitization and the slight anemia did not seem sufficiently severe to be responsible, in themselves, for the development of shock. While conducting this study we observed patients with more than 20 per cent parasitization of the peripheral blood cells and an erythrocyte count of barely 4 million who showed no evidence of shock, the hematocrit and blood pressure remaining essentially the same throughout the disease. Most of our shock cases showed greater anemia and some showed equal parasitization on the day after recovering from shock. The significance of intravascular clumping of erythrocytes in the production of anoxemia, as has been observed by Knisely, Stratman-Thomas, and Elliott (23) in monkey malaria, has not been determined but this phenomenon is known to occur in the late stages of shock.

3. The demonstration of hemoconcentration early in the development of the shock syndrome (Case 2) suggests that loss of plasma alone may be the initiating factor. Plasma loss may follow damage to the capillary walls by the action of hemolyzed erythrocyte constituents such as proteins and potassium, or "toxic" agents of parasitic origin.

Additional laboratory studies of blood changes, particularly determinations of plasma volume, electrolyte balance, the specific gravity of blood and plasma, and the plasma proteins may help in elucidating the specific mechanisms which initiate medical shock in cases of algid malaria.

#### SUMMARY

Evidence, in the form of 6 case reports, has been presented that algid malaria is due to the development of medical shock. This concept provides the clinician with a whole group of well recognized therapeutic aids for the treatment of a serious and often fatal illness.

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# AN OUTLINE FOR TEACHING MOSQUITO STOMACH AND SALIVARY GLAND DISSECTION

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## INTRODUCTION

Various methods of dissecting female anophelines to determine the presence or absence of oöcysts of plasmodia on the stomach and of sporozoites in the salivary glands are in use. Numerous descriptions of these procedures have been recorded in the literature, many being modifications of the method outlined in 1908 by Stephens and Christophers and later by Barber. Others who have contributed to this aspect of malariology include MacGregor, 1928; Craig, 1909; Boyd, 1930; Barber, 1930; Barraud, 1934; Missiroli, 1934; Russell and Baisas, 1935; Barber and Rice, 1936; Christophers and Covell, 1936; Wilcox and Logan, 1941; Covell, 1941; Simmons and Aitken, 1942; Puri, 1942; Blacklock and Wilson, 1942; Svensson, 1943; and Geiman, 1944.

At the Army Medical School it has been necessary to teach mosquito dissection to large groups of individuals who have had little or no previous training in entomology. The size of the staff available for teaching this material has been limited. Therefore, several different dissection techniques have been tested in an attempt to determine the most suitable method under such circumstances. The following teaching outline has been found to be satisfactory and is presented in the hope that it may prove useful at other institutions where a similar problem exists. It should be emphasized that the techniques here outlined are not original, but instead represent a combination of certain features of previously described methods that have proven to be useful in the classroom.

## PROCEDURE

The successful teaching of mosquito dissection by this method requires a minimum of one preliminary lecture and two laboratory periods of at least two hours each.

1. The preliminary lecture should include a brief description of the anatomy of the mosquito to orient the entomologically untrained person. This may be done with the aid of lantern slides. Unnecessary morphological detail should be avoided.

2. In the first laboratory period a minimum of two freshly killed uninfected mosquitoes are provided for each student. These are used for both stomach and salivary gland dissections. While any species may be employed for stomach dissection, anophelines are preferred for salivary gland dissection. When these

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are not available, however, either *Culex* or *Aedes* may be substituted satisfactorily. The following demonstrations have been utilized in the first laboratory period:

Demonstration	Type of Preparation
Alimentary tract and ovaries.....	Permanent mount
Unstained stomach.....	Permanent mount
Freshly dissected stomach.....	Temporary mount
Head of mosquito with tag of tissue and salivary glands protruding....	Permanent mount
Freshly dissected glands.....	Temporary mount
Stained normal glands.....	Permanent mount

3. In the second laboratory period a minimum of two freshly killed infected mosquitoes are provided for each student. These may be obtained by feeding the mosquitoes on birds infected with avian malaria, or by cooperation with hospitals where induced malaria is used in the treatment of neurosyphilis. The demonstrations considered desirable for the second laboratory period are as follows:

Demonstration	Type of Preparation
Living gametocyte of <i>P. cathemerium</i> showing exflagellation <sup>4</sup> .....	Temporary mount
Stained gametocyte of <i>P. cathemerium</i> showing exflagellation.....	Permanent mount
Unstained infected mosquito stomach <sup>5</sup> .....	Permanent mount
Freshly dissected infected mosquito stomach.....	Temporary mount
Infected mosquito stomach with small oöcysts.....	Permanent mount
Infected mosquito stomach with large oöcysts.....	Permanent mount
Infected salivary glands under high dry objective.....	Permanent mount
Infected salivary glands under oil objective.....	Permanent mount
Freshly dissected infected salivary glands.....	Temporary mount

#### DIRECTIONS FOR DISSECTION

##### A. Equipment Needed:

1. Clean glass slides
2. Small square cover glasses (No. 1), 9-11 mm.<sup>6</sup>
3. Relaxing chamber<sup>7</sup>
4. Aqueous methylene blue in 0.85 per cent NaCl solution
5. Needles or dental probes
6. Fine forceps
7. 70 per cent alcohol
8. 0.85 per cent NaCl solution
9. Dissecting microscopes

##### B. Preparation for Dissection:

1. Kill mosquitoes with chloroform, carbon tetrachloride or tobacco smoke shortly before they are to be used.
  - a. They should not contain recently ingested blood. An engorged mosquito will show a dark area in the ventral abdominal region. Re-

<sup>4</sup> Can be demonstrated in canary blood by fresh drop examination after an increase in gametocytes has been noted.

<sup>5</sup> *Culex pipiens* or *C. quinquefasciatus* infected with *P. cathemerium* may be used if *A. quadrimaculatus* infected with a human strain is not available.

<sup>6</sup> Can be cut from 18 or 22 mm. square cover glasses with a diamond point.

<sup>7</sup> Petri dish containing moist blotting paper will suffice.

cently caught anophelines should be kept in tubes with moist cotton plugs or in "mosquito hotels" for 48 hours, thus allowing time for digestion of a blood meal. Should it be necessary to examine a recently engorged mosquito, the blood may be removed by pricking the abdomen and then applying gentle pressure.

- b. The abdomen should not be distended by fully developed ovaries. If the specimen is gravid, the white ovaries may be seen shining through the abdominal wall.

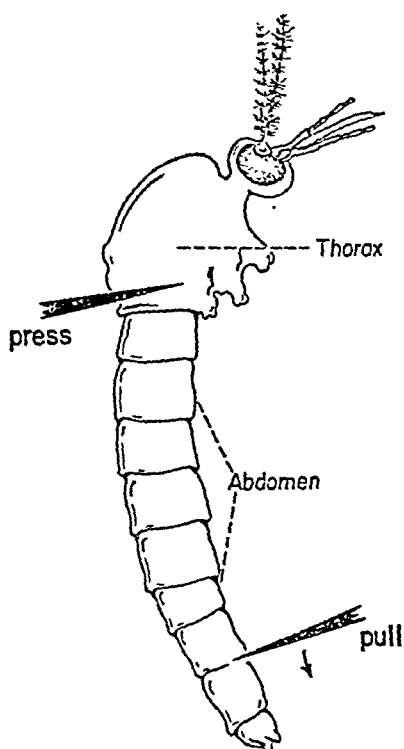


FIG. 1

FIG. 1. Position of needle in nicking abdomen of mosquito

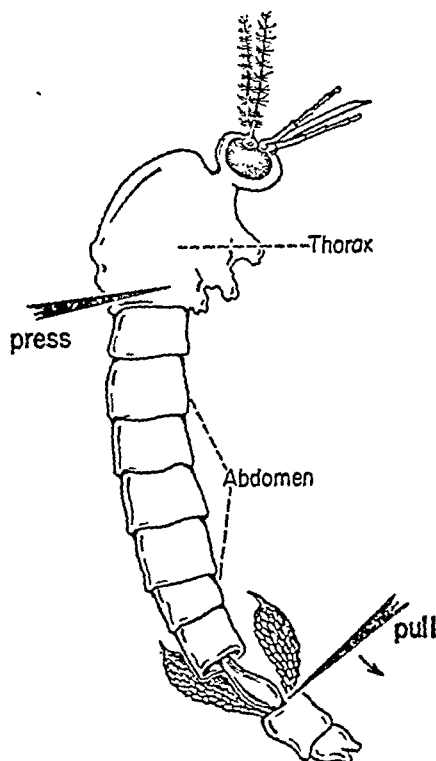


FIG. 2

FIG. 2. Technique of removing stomach

2. Identify to species.
3. Remove legs and wings, and place body with abdomen touching a drop of saline solution on a clean slide under a binocular dissecting microscope. Some workers prefer to dip the mosquito in 70 per cent alcohol before dissecting.

#### C. Dissection of Stomach:

1. Orient the body with the abdomen pointing toward you.
2. Hold the mosquito by transfixing the thorax with a needle at a point close to the junction of the abdomen with the thorax.
3. With a second needle nick the integument on both sides of the next to the last abdominal segment so as to partially sever the chitinous wall (Fig. 1).

4. Place the free needle on the partially separated terminal abdominal segments and by gentle intermittent traction draw out the stomach and the attached Malpighian tubules and ovaries (Fig. 2). If the gut does not pull out readily, and appears about to break, cease the traction and gently prod the posterior portion of the thorax with the free needle; this may aid in severing the anterior end of the gut.
5. Set aside the remainder of the mosquito in saline solution for salivary gland dissection.
6. Before examining the stomach for oöcysts, place a cover glass on the viscera and identify the following structures (Fig. 3):
  - a. The large stomach which is expanded posteriorly.

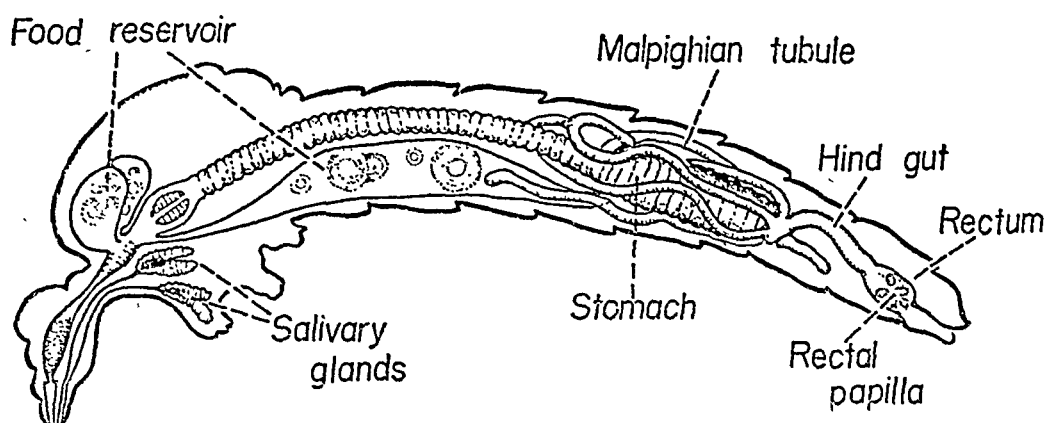


FIG. 3. Diagrammatic side view of mosquito showing internal organs with ovaries omitted (modified from various authors)

- b. The *Malpighian tubules* attached near the posterior end of the stomach. These usually appear as strings of "small sausages" extending anteriorly and overlying the stomach.
  - c. The *intestine* ending in a large *rectum* with indistinct *rectal papillae*.
  - d. The *ovaries* (See Fig. 2). In gravid females much of the abdominal cavity may be filled with developing eggs.
7. Prepare the stomach for examination for oöcysts as follows:
  - a. Remove excess saline with filter paper.
  - b. Carefully sever the gut posteriorly to the stomach and transfer stomach to a fresh drop of saline on the same slide, discarding attached Malpighian tubules, intestine, ovaries, and accumulated debris.
  - c. Gently lower the cover slip onto the stomach.
8. Examine the stomach wall for oöcysts.
  - a. Observe the network of branching tracheal tubules ramifying over the organ.
  - b. Note the deeper muscular layers.
  - c. Using the "high dry" objective search the outer layer containing the tracheal tubules for protruding oöcysts which appear as follows (See Figs. 7-10):

- (1) *Young oöcysts*: These are clear round bodies that are more refractile than the stomach cells and contain minute pigmented granules. They measure 6 to 12 microns in diameter.
- (2) *Older oöcysts*: These are more opaque than the stomach wall, contain distinct clumps of pigment, and measure 12 to 40 microns in diameter.
- (3) *Mature oöcysts*: The mature oöcysts measure 30 to 80 microns in diameter. They appear finely striated and contain enormous numbers of refractile spindle-shaped sporozoites that are 12 to 14 microns in length. Pigment granules are not present in the mature oöcysts.

*Caution*: Since small protrusions of the stomach membrane or fat cells may closely resemble immature oöcysts, make sure that the pigment granules characteristic of the immature stages are present. The large oöcysts are unmistakable.

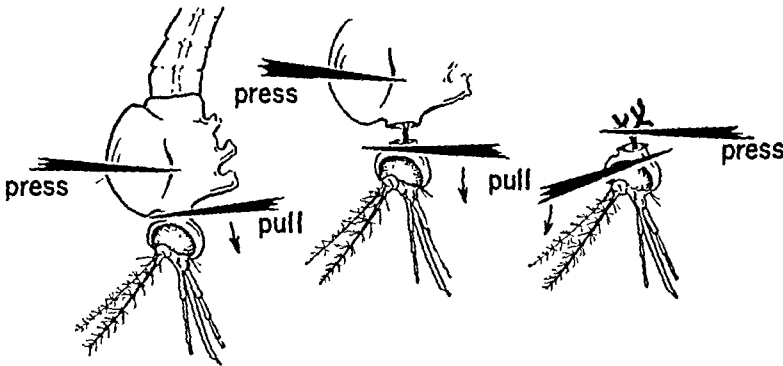


FIG. 4

FIG. 5

FIG. 6

FIGS. 4-6. Steps in removing salivary glands (modified from Blacklock and Wilson, 1942)

#### D. Dissection of the Salivary Glands:

1. Place mosquito (or the head and thorax if they were saved) in a drop of saline stained light-blue with methylene blue and proceed as follows:
  - a. Arrange mosquito on its side with the head directed towards you.
  - b. With the side of a needle exert gentle and continuous pressure on the anterior part of the thorax so that the neck bulges slightly (Fig. 4).
  - c. At the same time place the other needle behind the head and gently pull the head away from the thorax (Fig. 5).
  - d. As the head tears free from the thorax a small tag of tissue will be seen attached to the head. This tissue should contain the salivary glands (Fig. 6).
  - e. Transfer the head and attached tissue to a fresh drop of saline.
  - f. Isolate the salivary glands:
    - (1) Using the dissecting microscope look for the glands in the attached tissue. They are a pair of small, trilobed, refractile, faintly blue-staining bodies (Fig. 11). *Caution*: The glands may be easily overlooked since they may be partially hidden in the tag of tissue.

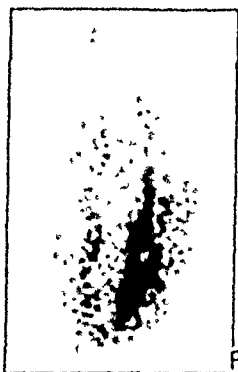


Fig 7

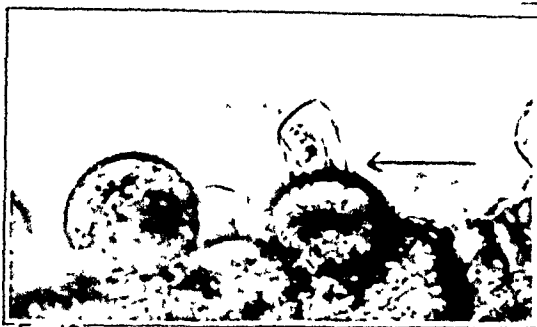


Fig 10



Fig 8

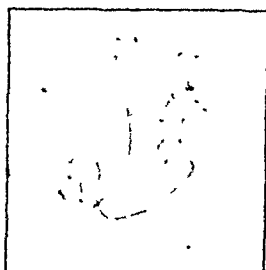


Fig 11

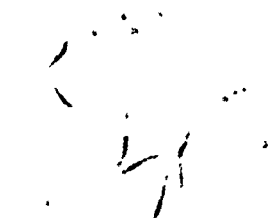


Fig 13



Fig 9



Fig 12

FIG. 7. Stained stomach showing an unusually heavy infection of oocysts (*P. vivax*).

FIG. 8. Unstained oocyst *P. vivax*. Notice Malpighian tubules.

FIG. 9. Oocyst containing developing sporozoites.

FIG. 10. Empty oocyst capsule

U. S. Army Medical Museum, Neg. No. 43048.

FIG. 11. Freshly dissected salivary glands, unstained, lower power.

FIG. 12. Salivary gland unstained showing sporozoites; high power.

FIG. 13. Sporozoites, Giemsa's stain (*P. vivax*).

- (2) Tease the glands free from the tissue.
- (3) Discard the debris and then mount the glands under a small cover glass (9-11 mm.).
- (4) Examine the salivary glands (Figs. 11, 12) and the surrounding region carefully under low and high dry objectives for the characteristic spindle-shaped sporozoites. Then crush the glands by applying pressure to the cover slip and examine.

- (5) Remove the cover glass and allow the material on it and on the slide to dry. Fix both preparations in methyl alcohol and stain with Giemsa's stain (Wright's stain may be used, omitting fixation).

If desired, the cover slip may be mounted smear side up on the slide. Examine with the high dry and oil immersion lenses for sporozoites, which appear as slender blue-staining spindles with a central red chromatin dot (Fig. 13).

#### DISCUSSION

It should be borne in mind that the methods outlined above are recommended for beginners only, and it is expected that such a procedure will, of necessity, be modified in the field as the worker becomes more proficient in making dissections. This will be essential if an attempt is made to determine the percentage of salivary gland positive mosquitoes in a given locality.

#### ACKNOWLEDGMENTS

The constructive suggestions of the personnel of the Division of Parasitology, especially Col. Paul F. Russell, M.C., of the members of several classes at the Army Medical School, of Dr. Mark F. Boyd, Director, and Miss Lucille Logan, Microscopist, of the Station for Malaria Research, Tallahassee, Florida, are gratefully acknowledged. The photomicrographs for figures 7, 8 and 13 were kindly furnished by Dr. Boyd. The aid of the artist, T/4 W. L. Brudon, A.U.S., Art Department, Army Medical School is gratefully acknowledged.

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# TSUTSUGAMUSHI DISEASE (SCRUB OR MITE-BORNE TYPHUS) IN THE PHILIPPINE ISLANDS DURING AMERICAN RE-OCCUPATION IN 1944-45<sup>1</sup>

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Investigations considered in this report began with the first appearance of scrub typhus in American forces after the landings and re-occupation of the Philippine Islands in 1944-45. Cases were recorded in December 1944 by medical officers recognizing the disease as it was contracted by combat infantrymen fighting through the Leyte Valley on Leyte Island. Observations beginning at that time continued until August 1945 during which period 241 cases of scrub typhus are known to have occurred in American Military personnel throughout the Philippine Archipelago.

Experience in New Guinea and adjacent islands reported in collaboration with the Surgeon SWPA, (Kohls et al (1945), Blake et al (1945) and others) with tsutsugamushi disease served not only to alert army medical personnel to the hazards of this mite-borne typhus-like disease but likewise thoroughly acquainted our medical authorities with the diverse epidemiological problems involved. Information on dangerous terrain, types and habits of the mite vector and control by camp area clearing and clothing impregnation were contributions of the early investigators in the South Western Pacific Area. The identification of scrub (mite-borne) typhus with tsutsugamushi disease as seen in Japan leads us to the use of these terms interchangeably in the subsequent discussion.

It is remarkable that incontrovertible evidence of tsutsugamushi disease in the Philippine Archipelago was not forthcoming until its occurrence in troops during military operations in the present war. In view of its present appearance in the coastal areas of the southern peninsula of Samar, we feel that the cases described by Ashburn and Craig (1908) from an Army camp (Connell) on the west coast of Samar in January of that year were probably instances of the disease. Febrile courses in both of approximately 2 weeks with rashes of several days duration, adenopathy, negative Widal's and malaria smears, with leukopenia all contribute to this probability in spite of absence of the typical eschar. Both authors had seen the classical disease in Japan and the senior author observed these patients clinically. It is true that the evidence presented does not preclude the possibility of infection with murine typhus. Foster (1912) four years later in a clinical study presented evidence that "endemic typhus" was

<sup>1</sup>Presented at the Forty-first Annual Meeting of the American Society of Tropical Medicine, November 13-15, 1945.

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occurring on the Island of Mindanao, but he suspected transmission by the head louse.

De Roda (1937) published the results of a serological survey of blood specimens from a group of 500 patients hospitalized in Manila with the diagnosis of influenza, typhoid fever and tuberculosis. Although not attempting to differentiate between murine and tsutsugamushi disease his study employing one or more of the three proteus organisms (OX19, OX2, OXK) revealed high titers with the sera of 47 of the cases. In this group of patients showing high proteus agglutinins 3 died, among whom 2 had the post mortem findings of typhoid fever and one of tuberculous meningitis. De Roda selected a titer of 1/500 as a significant diagnostic level since surveys of sera submitted for the Wassermann and other reactions often revealed agglutination of 1/100, 1/200, and rarely 1/500. High titers (over 1/500) for OXK, OX19, and OX2 occurred in 5 different individuals and of his serologically significant OXK patients 5 were of urban and 7 of rural origin. The clinical course of the disease is not specifically presented for these individuals. His work nevertheless suggests that some form of typhus fever was being encountered.

In World War II during the Japanese occupation of the Philippines, cases apparently occurred in their forces on Mindanao as recorded in ADVATIS Bulletin No. 64 (14 Dec. 1944). Reference to the original captured medical document in Japanese gives no specific location and refers only to an unknown febrile infection resembling Japanese River fever with rash, adenopathy and positive Weil-Felix (type unstated, but presumably OXK with which certain of their field laboratories were known to be familiar). In United States Army forces on Mindanao only 2 cases of rickettsial disease became known to us. One was proved serologically to be murine (flea-borne, OX19) typhus and the other, listed later, appears to have been a case of tsutsugamushi disease contracted during combat. The exact locality of the exposure to infection could not be determined with certainty. Foci in which infections have been contracted in American troops have appeared during operations on the Islands of Leyte, Samar, Mindoro, Luzon, Negros and Mindanao. Presence of tsutsugamushi disease on each island has been confirmed by clinical and laboratory studies, and by the recovery of the etiological agent in white mice from a patient on Samar, as detailed below, and the conditions under which infection took place in most localities have been explored.

#### IDENTIFICATION OF THE DISEASE

Recent clinical studies undertaken by various military observers since the advent of the Pacific War augment nicely certain features lacking in much of the prior classical descriptions of scrub typhus. In a recent monograph Blake et al (1945) have exhaustively reviewed the voluminous literature and added their own clinical and laboratory observations on patients in New Guinea. Since that publication Levine (1945) has evaluated the cardiac and general circulatory disturbances in this disease. His bedside observations coupled with electrocardiograms, measurements of vital capacity of the lungs, roentgen examination of the hearts,

exercise tolerance tests, and determinations of the Schneider index during early convalescence added valuable information. The evidence which he presented indicated that residual myocardial damage, per se, following scrub typhus infection is extremely uncommon. Howell, (1945) utilizing a group of 200 cases observed somewhat earlier in the course of the infection confirmed these findings. Our observations on 20 patients employing when feasible electrocardiograms, blood pressure readings, exercise tolerance tests and bedside studies support their conclusions. Gottfreid (1945) and others have investigated various physiological and blood chemical changes. The lowering of plasma proteins with a reversal of the albumen-globulin ratio is now well recognized in severe cases. Deficient quantities of fibrinogen and a rather pronounced hypochloremia during the acute febrile phases are significant physiological alterations.

TABLE I  
*Incubation period of a group of cases: Bicol Peninsula, Luzon*  
Beach Landing on April 29, 1945

CASE NO.	DAYS ON BEACH (EXPOSURE)	ONSET OF ILLNESS	RANGE OF INCUBATION IN DAYS	ADMITTED TO HOSP.	CONFIRMATORY SEROLOGY	
					OXK	C. Fix.
1	Apr. 29-May 3	May 9	5-10	May 12	1/160	1/32768
2	Apr. 29-May 2	May 13	9-14	May 17	1/320	1/4
3	Apr. 29-Apr. 30	May 14	13-15	May 16	1/160	1/4096
4	Apr. 29-May 5	May 15	9-15	May 21	1/320	1/32768
5	Apr. 29-May 1	May 16	14-17	May 20	1/640	1/512
6	Apr. 29-Apr. 30	May 16	15-17	May 18	1/40	1/256

We will merely present briefly the characteristic clinical features of the disease as observed in these soldiers contracting it in the Philippines. Detailed descriptions of the clinical course in individual cases are not presented since they differ in no essential respect from those given by previous authors.

#### *Clinical Observations*

Our most reliable data on incubation were obtained from a small unit outbreak occurring in landing forces on southern Luzon in April 1945. The infecting area was localized to a section of sandy beach overgrown with coconut palm cover not over 300 yards in length and 200 yards in depth. Assuming that infection was probably acquired on this beach strip, Table I indicates a maximum range of 5 to 17 days incubation, a figure coinciding in general with other known localized episodes of scrub typhus throughout the South West Pacific, the shortest of which was 7 days (Kohls et al, 1945).

Cases of scrub typhus with a typical clinical picture have been encountered on each of the Philippine Islands considered in this study. Lymph glandular enlargement has been an almost universal feature with early and marked reaction of the satellite glands in the region of the eschar. The location of the eschar has been variable. It has been observed and recorded in fully 40 per cent of all the

Philippine cases, while on Mindoro over 65 per cent presented this sign. Multiple primary lesions were reported in 2 instances. A maculo-papular rash was recorded in just 50 per cent of the cases on whom some clinical data was available. The rash has varied from a fleeting morbilliform eruption to a maculo-papular petechial lesion of 4 to 8 days duration. In the seriously ill patients evidence of general circulatory embarrassment with cyanosis, tachycardia, lowered arterial tension and dyspnoea usually developed. In many of these same cases evidence of central nervous system involvement with delirium, deafness, tremors, and frank convulsions were also observed.

A continuous febrile course ranging from 10 to 21 days has been the rule. Pyrexia of 103 to 105°F. during the second week of illness was not uncommon. Intense headache with general malaise, weakness, anorexia, and nausea have been

TABLE II

*Salient clinical and serological data on the Philippine cases listed for each island*

ISLAND	NUMBER OF CASES	RASH	ESCHAR	DEATHS	WEIL-FELIX REACTION	
					Positive OXK*	Rise in Titer
Mindoro.....	95	15	64	6	34	6
Samar—Army....	22	7+	7+	2	9	3
Navy.....	66*			0	66	
Luzon.....	28	19	9	2	23	14
Negros.....	7	3	7	0	4	2
Leyte.....	3	3	2	0	2	
Mindanao.....	1		1	0	1	
Totals.....	222	47	90	10	139	25
Percentages....		21.2†	40.5†	4.5	62.6	11.3

\* Agglutination of standard suspension in serum dilutions greater than 1:80.

† Clinical observations under field conditions did not always permit careful examination of these patients for rash, and eschar.

\* Incomplete.

frequent complaints during early and acute phases. Conjunctival injection with suffusion have been constant. The pneumonitis so frequently observed in scrub typhus has not responded to either penicillin or chemotherapy. The frequency of splenomegaly was uncertain.

For purposes of simplicity the significant clinical and laboratory findings are tabulated below (Table II).

### *Pathology*

Lt. Col. William B. VanderGrift of the 118th General Hospital performed a post mortem examination on the two fatal Samar cases. His summary of the pathological findings is as follows: "Lesions were extremely widespread and involved practically all organs, including skeletal muscles and loose connective tissue. Spleens and lymph nodes were slightly to moderately enlarged. Myo-

cardium of each case was diffusely mottled with tiny grayish-pink foci. Uniform, light gray, translucent consolidation involved most of the lungs in each case. Brains were hyperemic and edematous. Petechial hemorrhages were inconspicuous. The outstanding microscopic finding was numerous mitotic figures in the plasma cells, which were the most prominent inflammatory cell. Vasculitis was a rare finding; no vascular nodules were found. In each case the following changes existed; acute splenic tumor, lymphadenitis, tracheitis, bronchitis, bronchiolitis, interstitial pneumonitis, myocarditis, meningitis, encephalitis, swelling of connective tissue cells and infiltration of practically all organs and soft tissue with plasma cells, macrophages and lymphocytes. In addition, one case showed many poorly defined necroses and occasional 'typhus nodules', fibrinous alveolitis and localized atrophy of the upper part of the right lobe of the liver."

Major J. D. Winslow, Pathologist of the 26th Medical Laboratory, in his microscopic analysis of the 2 Luzon fatalities reports focal infiltration by lymphocytes and mononuclear cells in the heart, lungs, liver, kidneys and gastrointestinal tract. Minute areas of focal necrosis and hemorrhage with moderate perivascular cuffing by round cells were observed in both cases.

### *Mortality*

The overall mortality on the basis of 10 deaths among 222 cases of scrub typhus occurring among service personnel in the Philippines is 4.5 per cent. From Mindoro there were 6 deaths in 95 recorded cases. The Navy records no deaths among 66 serologically proven patients on Samar in comparison to 2 fatalities among 22 army personnel in that area. There were no fatalities from Leyte or Negros although 2 deaths among the 28 Luzon cases are registered. Undoubtedly prompt hospitalization and good medical treatment contributed to the low fatality rate as well as the age and physical condition of American military personnel.

### *Laboratory Findings*

The serological findings confirmed the clinical evidence. High diagnostic titers of proteus OXK agglutinins were demonstrated by the Weil-Felix test. In many instances a rise in titer was found by spaced examinations of blood specimens in series from the same individual. Antibodies of scrub typhus were also demonstrated by a complement fixation test.<sup>5</sup> In Table III the serological results on specimens from the Luzon cases are presented. This was our most carefully studied group of patients. In general the high OXK titers appeared during the latter part of the second week of illness and throughout the third and fourth weeks.

<sup>5</sup>The complement fixation tests were performed by Dr. Ida Bengston, National Institute of Health, U.S.P.H.S. The antigen employed in this test will be described in a subsequent publication.

*Isolation of Etiological Agent*

A virulent strain of *Rickettsia orientalis* has been successfully isolated by the authors from a patient who contracted scrub typhus on the Island of Samar. The origin of this strain was blood taken on the 11th day of illness from an acutely ill patient (J. H. V.) whose condition at the time of venipuncture was complicated by a bilateral pneumonia. Both an eschar and a generalized

TABLE III

*Results of Weil-Felix and complement fixation tests on sera from 28 Luzon cases*

CASE NO.	Onset	WEIL-FELIX PROTEUS OXK				COMPLEMENT FIXATION	
		Day	Titer	Day	Titer	Day	Titer (Karp ant.)
1	Jan. 15	8	1/320	34	1/80		
2	Dec. 28	5	1/640	17	1/1280		
3	Feb. 15	12					
4	Jan. 25	15	1/160				
5	Feb. 16	30	1/320				
6	Feb. 28	Not available				16	1/8192
7	Apr. 18	16	1/80	26	1/2560		
8	Apr. 22	Fatal case. Died May 4 No serology					
9	Apr. 28	12	0	25	1/320	25	1/64
10	Apr. 30	20	1/320	29	1/1280	29	1/2048
11	May 4	14	1/160	20			
12	May 6	13	1/20	23	1/320	23	1/128
13	May 6	10	1/20	24	1/160	24	1/2048
14	May 7	9	1/320	23	1/80	23	1/128
15	May 9	21	1/80	33	1/160	33	1/32768
16	May 11	Not available					
17	May 13	18	1/80	30	1/320	30	1/4
18	May 14	17	1/160	29	1/160	29	1/4096
19	May 15	16	1/80	28	1/320	28	1/32768
20	May 15	13	1/160	25	1/320		
21	May 16	15	1/80	27	1/640	27	1/512
22	May 16	15	1/20	28	1/40	28	1/256
23	May 17	14	1/80	26	1/320	26	1/4096
24	May 25	18	1/1280				
25	May 27		1/80	16	11/1280	16	1/16384
26	June 1	12	1/80			12	1/256
27	June 10	6	0	Fatal case. No further serology			
28	June 15	Not available					

maculo-papular rash occurred in this individual whose OXK titer at time of withdrawal of blood was 1/160. Subsequent examination revealed a final titer of 1/1280 on two separate specimens in series.

Two white mice inoculated intraperitoneally died on the 12th and 14th day respectively and the remaining 2 moribund animals were sacrificed on the same named days for passage. *Rickettsiae* were observed in preparations made from peritoneal exudate and in splenic impression smears after the second passage.

Now in the 7th generation, death appears in mice after 8 to 10 days. Viscid peritoneal exudate, splenomegaly, pleural effusion, and enlarged inguinal glands are consistent features.

Capt. Byron Bennett, SnC<sup>6</sup> in further laboratory experiments with this Samar virus has successfully demonstrated its reciprocal cross protection with New Guinea and other identified scrub typhus strains.

## EPIDEMIOLOGY

### *Geographic Distribution*

Any attempt at comparison of intensity or distribution of infection between islands is naturally weighted by the numbers and conditions of exposure of military personnel and of clothing impregnation in combat units. Apparently the most widespread foci have been on Luzon, though the total number of cases on this Island is less than on either of the single foci found on southeastern Samar (Guinan area) or southwestern Mindoro (San Jose area). A map showing geographic location of the various foci in the Philippines is presented in Figure 1.

The major islands which have so far remained without report of infection with this disease are Palawan, Panay, Cebu, Bohol and Masbate. It is notable, however, that on these islands the number of men engaged in military operation was comparatively small. It seems highly probable that a careful field study would reveal the presence of the infection on these islands as well.

### *Seasonal*

In general, wet and dry seasonal differences are more pronounced on the western sides of most of the Philippine Islands than on the eastern (Global Epidemiology 1944). The exigencies of troop movement and operations providing variable exposure in the different areas, plus the relative shortness of time in location, made it impossible to evaluate satisfactorily any seasonal effect on incidence. Judging from previous experience with scrub typhus under relatively comparable climatic conditions in New Guinea, one would not expect any approach to the marked seasonal occurrence reported in northern Japan.

The epidemic in southwestern Mindoro was coincident with the dry season, but also with the maximum exposure during initial operations. There is no marked seasonal variation in climate in southeastern Samar where the other outbreak of consequence occurred, though the period of maximum occurrence was reported to have been approaching the driest stretch of weather in 11 years.

### *Description of Foci: (Ecology)*

The environmental features encountered in the several foci where sources were definitely traceable, have not been as widely variable as in our New Guinea experience (Kohls et al, 1945). There are, however, two that are roughly comparable. The Guinan area of Samar is predominated by a coconut grove canopy underlain

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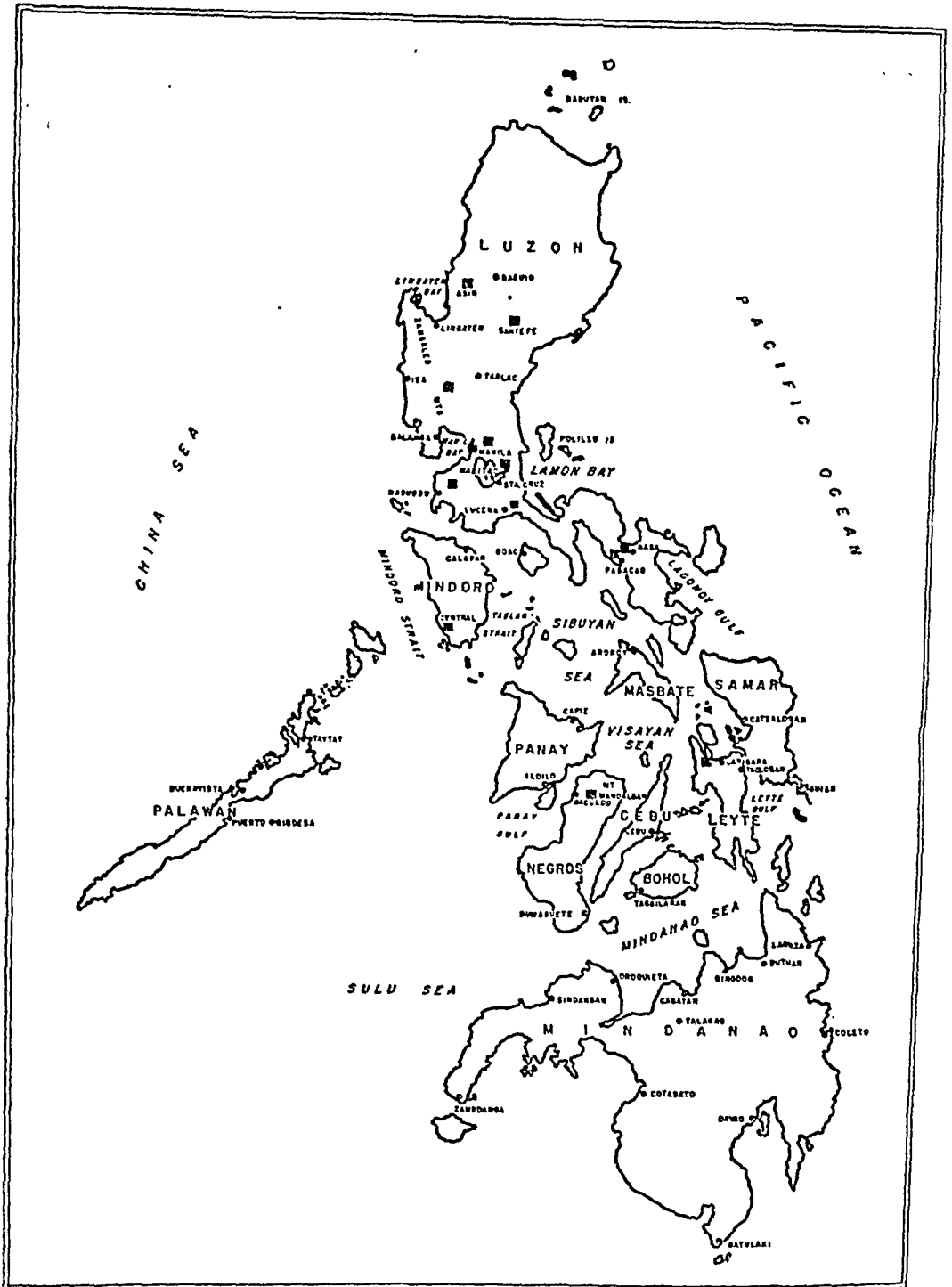


FIG. 1. Foci of scrub typhus Philippine Islands

by a dense secondary growth of scrub undoubtedly developed following enforced neglect during Japanese occupation. The rough vegetative cover bore striking resemblance to focal areas seen at Owi, Biak and Middleburg Islands, and Samsapor beach margins. The grassy flats and slopes of the Mindoro and Negros



focal areas also were reminiscent of the kunai grass fields of British New Guinea where localized outbreaks of scrub typhus occurred.

1. *Samar*. Scrub typhus in American Forces first appeared in naval units in the Guinan area of this island in early December 1944. The disease built up to proportions of a small epidemic, (see Table II) a third of which were in scattered Army units during January and early February, and sporadic cases have continued to appear into July of 1945. The local strength fluctuated especially among Army units, but Naval personnel predominated at this base through the period. Localized foci were not apparent, and affected units were well scattered including adjacent Calicoan Island.

TABLE IV  
*Distribution of cases by locality and month of occurrence*

ISLAND LOCALITY	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	TOTAL
Mindoro (San Jose) D-Day Dec. 15, 1944.....			13	67	9	2	4				95
Samar (Guinan) D-Day Oct. 24, 1944..				32	31	20	4	4	5	1	97
Luzon											
(1) Bicol, April 29, 1945.....								10			10
(2) Baguio, Undet.....							2	3			5
(3) Santa Fe, Undet.....								1	2		3
(4) Batangas, Undet.....					1			1			2
(5) Scattered.....				2	3		2	1			8
Negros (Mt. Mandalagan) D-Day March 29, 1945.....							3	3	1		7
Leyte (Leyte Valley) Oct. 20, 1945....			1	2							3
Mindanao (75 mi. NW of Davno) April 17, 1945.....										1	1

Total 241

All traceable infections originated in units installed among the universal coconut groves described above. The undergrowth and ragged coral outcrops provided an abundance of rat cover. Surveys by various Naval control units indicated the early prevalence of both rats and mites. The indigenous rats comprised 2 species related to those seen on other islands, viz. a small species of the *exulans* group, and numerous *Rattus mindanensis*. At the time of our late visit, both the rats and mites were reduced in numbers, owing in part at least to active control measures. *Trombicula deliensis* was identified from rats trapped in the 5th Bomber Group area, and in mounts shown us by Lt. Mage of the 544 C.B. rodent control unit.

2. *Leyte*. The first of 3 cases in soldiers occurred 14 Dec. 1944. All 3 developed late in the campaign during fighting in Leyte Valley. They were subsequently hospitalized and the clinical diagnosis was confirmed serologically for two. It was not possible to trace the sources of infection and the type of environment remains obscure. No rat or mite surveys were made in Leyte Valley.

3. *Mindoro*. The largest outbreak among troops in the entire Philippine campaign occurred in the San Jose district in southwestern Mindoro. Extensive fields of waist to shoulder-high "talahib" grass had overgrown neglected cane fields in the whole district. There appeared to be 3 localized areas of infection, the first of which was encountered by the 503d Parachute Infantry near the mouth of the Bugsanga River where prone exposure among the grass was necessitated during early operations. The first case was hospitalized Christmas Day 1944, 10 days after initial landings. Another focus appeared near Hill Airstrip where several cases were undoubtedly contracted among 58th Fighter personnel while seeking shelter in adjoining grass during numerous air raids. The third area of infection was at the south section of the beachhead occupied by the 19th and 21st Infantry Regiments of the 24th Division.

An example of the characteristic localization of infection within extensive grassy areas, as previously seen in kunai flats in New Guinea, may be drawn from the field report to the Surgeon 8th Army by one of us (RRS). "The greatest number of cases, i.e. 25 (of 70 total at the time) occurred in the 503d Parachute Infantry, 15 of these in A Company alone. Of these, 6 occurred in the 3rd Platoon of A Company. Questioning of these cases disclosed the interesting fact that five of the six were in the same split squad. The other half of the same squad in which no cases occurred, and which operated separately was approximately 200-300 yards away at all times during the first 10 days of the operations on Mindoro." (i.e., the incubation period).

Rat and mite surveys were not instituted until 3 months after the initial cases when the dry season had set in making the dry soil conditions appear most unfavorable for mites judged by previous New Guinea experience. Rat burrows were more numerous in the loose friable soil of these previously worked cane fields than in any other area visited, and the rats were found to be correspondingly abundant. The burrows were dry and relatively shallow but there was a surprising number of mites in the ears of the trapped rats in spite of the dry conditions.

The rats were of 3 kinds, viz. *Rattus mindanensis*, *R. rattus umbriventer* and *R. vigoratus* (small *exulans* group) (ident. USNM). *Trombicula deliensis* was taken in moderate numbers on rats from several areas. Other mite species are listed later.

Attempted "boot collections" of mites were unsuccessful, probably accentuated by the dry conditions and no specimens off man had been saved from attacks early in the operations to enable identification. Whether any vectors were involved other than *T. deliensis* could not be determined.

4. *Luzon*. Of the 28 total cases no more than 6 were contracted in the same locality of this Island. They have been in scattered locations from the vicinities of Baguio and Sante Fe in the north to Pasacao on the Bicol Peninsula in the south as indicated in Figure 1. The Pasacao area, where 6 cases were contracted during 5 to 17 days exposure consisted of a level sandy floor just above the beach with a vegetative cover of low undergrowth and coconut palms resembling the Bat Island focus previously described (Philip and Kohls, 1945). A rat and mite survey here was not attempted.

A case originating at Mabitae, northeastern end of Laguna de Bay, was probably contracted while his unit was bivouaced in a pasture of short grass at the edge of town. *T. deliensis* was again found on rodents in this area. Whether the variability involved only this, or other related vectors too, is discussed in a later article. The pasture was bordered by brush beneath scattering coconut palms extending up onto nearby slopes, and into a creek bed on another flank. Good rat cover was thus available but indigenous rats were not as abundant as on Mindoro and Samar.

Several cases have developed during operations in mountainous districts. Three cases apparently originated during bivouacs on "Hill 3000" near Asin on the approaches to Baguio. Most of the mountains in the vicinity are covered with scrub and low brush, sparse trees, and with many bare grassy knolls and ridges on the crests. It is manifestly impossible to trace the exact sources of such cases where patrol work during the incubation period has been so variable under combat conditions. Nor has it been possible to make more than cursory surveys for rats and mites under these limited conditions. Mites referable to the vector group, *akamushi-deliensis*, have been taken from 2 species of rats of the *rattus* and *exulans* sections of *Rattus* in the Lingayen and Bagabag areas of Northern Luzon as amplified below.

5. *Negros*. Seven cases have originated during action on the lower slopes of Mt. Mandalagan near Bacolod. Though patrol sections embraced both grassy ridges and the woody and brushy areas of the mountains, questioning of the patients suggested that infections were acquired while the men were bivouaced on the upper grassy ridges near the forest margins. Two soldiers occupying the same foxhole on one grassy prong were hospitalized simultaneously. The dominant grass here was so-called "kogan" (*Imperator*).

Rats were extremely scarce in those areas, and no mites were taken during attempted boot collections. Only 2 rats of 29 *R. rattus umbriventer* trapped during 3 weeks of effort by Major L. D. Christenson of the 403d Malaria Survey Detachment, showed mite infestations of the ears. *T. deliensis* and *T. akamushi* represented 2 of the 3 species identified.

6. *Mindanao*. The one proven case was an infantryman who had been in combat near Namnan about 75 miles northwest of Davao.

### Rodents

It was not possible in the brief visits to the various areas to institute more than casual trapping surveys for local rodents. Because of previous experience, emphasis was placed on rats as the most likely "animal reservoirs" of scrub typhus. Help in these surveys often has been generously provided by various local Army units as acknowledged later. No attempt was made to recover strains of infection from indigenous rats by animal injection.

As implied above, the commonest rat encountered in various areas was the medium sized variety, *Rattus mindanensis*. This rat was abundant on Mindoro and Samar where their burrows were readily found and numerous. This was in contrast to the scarcity of locatable nests and burrows in New Guinea focal areas. Specimens of this form have been taken on Negros and Luzon. A

subspecies (*umbriventer*) related to this has been reported among specimens forwarded to the U. S. National Museum from Mindoro and Negros.

Widespread also is a small rat of the *exulans-concolor* group which is more prone to invade communities and houses but is usually less heavily infested with mites as observed so far. This form was taken on Mindoro, Samar, and several places in Luzon. Specimens from Mindoro were identified as *Rattus vigoratus*. Luzon samples of related forms taken by Captain Mohr were assigned to *calcis* (USMN). Relatives of both of these groups of rats probably will be found throughout the Archipelago, but as pointed out by the taxonomists on the basis of the preliminary surveys so far, further collecting will be required to determine to what extent biologically distinct races and subspeciation has occurred in different islands and localities.

The commensal rats, such as *Rattus norvegicus* occurring in abundance in coastal villages and cities, have not been found infested with important species of mites. An uninfested *norvegicus* was taken in a newly established Army tentage area on dirt floors 10 miles inland on Negros and completely isolated from any native village. This bordered an area where cases had occurred.

A subject we were unable to investigate but which may be of importance in faunal studies relating to this disease concerns local birds and their mite parasites. Vector species on avian hosts have been found previously, especially on ground frequenting species.

### Mites

Accepting Womersley's latest opinion (correspondence) of synonymy, we find 2 species of known importance as vectors of scrub typhus in the Philippine Islands. These are *Trombicula akamushi* (syn. *T. fletcheri*) and *T. deliensis* (syn. *T. walchi*). The first has been shown by several Japanese investigators to be a natural vector in Japan and confirmed by Blake et al (1945) as a vector in New Guinea. Natural infection in the second was first proved recently in the Admiralties in Bat Island mites (Philip and Kohls, 1945).

A later publication on Trombiculidae collected in the Philippine Islands will embody our complete observations of mites and rodents throughout the Archipelago in which paper a key to the Island mites will be presented. For purposes of this paper we will list only the mites collected throughout the period of combat operations. They are: *Trombicula akamushi*, *T. deliensis*, *T. wichmanni*, *T. acuscutellaris*, *T. bodensis*, *T. scincoides*, *Heaslipia (Trombiculoides) gateri*, *Neoschongastia indica*, *N. philippensis* (1946) and *N. kohlsi* (1946). *T. wichmanni* was found attacking man in Northern Luzon.

### SUMMARY

1. Cases of scrub typhus have occurred in troops during actions on the Islands of Leyte, Samar, Mindoro, Luzon, Negros and Mindanao. Japanese troops were reported to have encountered the disease on Mindanao. The largest epidemics occurred on Mindoro and Samar; widest distribution was encountered on Luzon.

2. The clinical and laboratory findings were in agreement with previous military experience with this disease. The case fatality rate was 4.5 per cent.

3. A strain of *Rickettsia orientalis* was recovered in laboratory mice from a patient on Samar and has been carried for 7 passages. Cross immunity with known strains of scrub typhus isolated in other geographic areas has been demonstrated.

4. As in previous experience, environments where infections have been contracted have varied. Focal areas have been encountered in fields of the common Philippine grasses, "talahib" (*Saccharum*) and "kogan" (*Imperator*), as well as neglected coconut groves with scrub undergrowth overlying both sandy and corraline floors. Most infections were contracted at or just above beach levels but some came from mountain scrub areas as high as 3000 feet. (Outbreaks appeared to be more referable to exigencies of military operations than to detectable seasonable influence.)

5. Rats indigenous to focal areas have been identified as *Rattus mindanensis*, *R. rattus umbriventer* and a small rat of the *exulans* group, *R. vigoratus*. The first appeared to be the most important mite host.

6. *Trombicula deliensis*, a known mite vector was taken from rats of all the above islands except Leyte. The other proved vector, *T. akamushi*, was identified from rats on Luzon and Negros. Eight other species of mites have been found on Philippine rats.

7. Incontrovertible evidence of the presence of tsutsugamushi disease (scrub typhus) in the Philippine Archipelago has been forthcoming for the first time during military operations on several islands in 1944-45.

#### ACKNOWLEDGEMENTS

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Chiefs of medical services and ward officers throughout the Philippines performed the bulk of the bedside observations and aided in the collection of statistical data. In this group we wish to acknowledge specifically the 117th Station, the 71st and 36th Evacuation and the 118th, 44th, and 49th General Hospitals.

To Lt. (SG) Lawrence W. Kinsell MC, USNR and Comdr. Ellis W. Willhemy, MC, USNR of the 114th Fleet Hospital we are grateful for their permission and assistance in the limited laboratory procedures performed on cases from their wards which resulted in the successful isolation of a typhus strain in animals.

For field and laboratory facilities in addition to aid in collection of scientific data it would be a distinct pleasure to name officers and men individually of the medical installations concerned if space permitted. In citing the 26th and 3d Medical Laboratories, the 5th, 26th, 31st, 58th and 403d Malaria Survey Detachments and the 4th Medical Museum Arts Detachment, we express grateful appreciation to those men who participated in this work.

Identification of rodents was provided through courtesy of Dr. Remington Kellogg of the U.S. National Museum. Samples of Philippine grasses were named by Dr. Eduardo Quisumbing, Botanist of the Philippine Bureau of Science.

Dr. Kenneth F. Maxcy, Consultant to the Secretary of War and member of the Army Epidemiological Board gave much helpful assistance in preparation of the text.

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# A CONTRIBUTION TO OUR KNOWLEDGE OF THE BIONOMICS OF THE COMMON NORTH AMERICAN CHIGGER, *EUTROMBICULA ALFREDDUGESI* (OUDEMANS) WITH A DESCRIPTION OF A RAPID COLLECTING METHOD<sup>1</sup>

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Chiggers, the larval form of certain mites, have long been recognized as a pest having a great nuisance value since their bites cause a violent itching and, in severe attacks, have caused febrile symptoms. But today, because of conditions arising from the present world conflict, they have become not only a pest but a true natural bottleneck in our wartime program. Among the armed forces of our country thousands of man hours of sleep have been lost with a consequent drop in efficiency. Many more hundreds of man hours have been idled away in hospital bunks because of secondary infections derived from scratching and infecting the bites made by these hexapod parasites. In the Southwest and Central Pacific, as well as other regions of the Far East, is found a group of chiggers of the genus *Trombicula*, closely related to our own common chigger, which is transmitting a disease, scrub-typhus, to our military forces. Thus it behooves us, today more than ever before, to possess a complete knowledge of the bionomics of these parasites.

## COLLECTING CHIGGERS IN NATURE

### *Where to collect*

In the course of the chigger study it was imperative that large numbers of these larval mites be collected in the field. It was first necessary to find locations where they could be collected in abundance. Two types of localities proved to be typical haunts of our common "red-bug," *Eutrombicula alfreddugesi* (Oudemans). They were to be found in greatest numbers under and near blackberry bushes. Although not quite as prevalent, they could also be found in relatively large numbers near the base of pine trees, particularly at the base of those that were in groups of three or more. The numbers collected at various other types of localities throughout wooded and semi-wooded areas were most meager in comparison.

In attempting to explain phenomenon of nature the simplest explanation frequently is the correct one. Ewing (1929) states that "It would appear that in many localities ground-frequenting birds are of more importance as hosts than

<sup>1</sup> The topics herein discussed represent a portion of the work conducted on chigger research between June 1, 1942 and February 28, 1943, at the School of Public Health, University of North Carolina, Chapel Hill, North Carolina. This research was financed by the Eli Lilly Company through Dr. H. W. Brown to whom I am greatly indebted for his helpful suggestions.

either the rabbit or box-turtle." Therefore, it seems to me that perhaps the simplest explanation for the largest number of chiggers inhabiting the environs of blackberry bushes is to be found in the fact that ground-feeding birds and perhaps certain rodents, which ordinarily are distributed over relatively large areas, are attracted to these spots, which are relatively small in area, to feed during the berry season. While some of these animals are frequenting the berry patches, chiggers which have become engorged on their epidermal cells detach themselves and drop to the ground to continue development, as is their habit when they become fully engorged. Thus, over a given period of time, the same number of "red-bugs" will have dropped to the ground over a small area as would have dropped out over a larger area had the birds not come to feed upon the berries. It would require only twenty-five females to produce the 10,000 chiggers which I collected under one group of bushes during the summer, providing they all lived, and each gave rise to 400 offspring.

#### *When to collect*

Chiggers were found in abundance, in the latitude of Chapel Hill, Orange County, North Carolina, from the first week in June until the last few days in August. Although they occur in numbers from June through August they no doubt make their first appearance before that date and most certainly can be found after August. Several were found at my usual collecting spot on October 18. On October 29, after several nights of frost, none could be found, nor were any observed on November 16, when the temperature suddenly rose to 75°F.

These hexapod larvae were much more prevalent and much more active in the afternoon than in the forenoon. The best hours for collecting appeared to be between 1:30 and 4:30 P.M. They seemed to be nearly as numerous after a rain as on a bright sunny day.

#### *How to collect*

Several standard methods of collecting the chiggers were attempted. However, it soon became apparent that a new method would have to be devised if the larval mites were to be collected by the hundreds. The most satisfactory, from the standpoint of time and numbers obtained, of several new methods which were tried out, was what I will term the "white dish" method. This method was modified from one used by workers at Duke University a year or two before (unpublished). It consisted of placing a number of white saucers, usually from five to eight, where the chiggers were found in greatest numbers, i.e. under or near the blackberry bushes. These saucers were placed several feet apart. Upon examining a saucer after a minute's exposure to the grass upon which it rested, several minute red specks could clearly be seen moving about rapidly over the white background. These chiggers were "sucked" into an aspirator, constructed from a glass vial or test tube, a two-hole stopper, two small pieces of glass tubing, and two short sections of rubber tubing. Thus it was possible to collect them rapidly and bring them back to the laboratory alive. Usually more would be found on one saucer than upon the others indicating a greater density of



chiggers in that area. All the saucers were then placed adjacent to the one having a high count. The chiggers were collected from one saucer, after which it was placed in its original location and a second saucer examined, etc. By the time all the saucers had been examined once, the one upon which the initial examination had been made would have more "red-bugs" scampering about over its surfaces. Several hundred would frequently be found in a small area of about eighteen inches square, while six inches away only three or four, if any, could be collected. This would seem to indicate that the larval mites do not migrate far from the original location of the egg mass.

There were indications that large white enameled pans would be even more satisfactory than the white saucers. However, these were not used until the chigger season was decidedly on the wane and it would therefore be difficult to justly compare the two utensils as to effectiveness at this time.

Judging from the literature and from conversations I have held with individuals who have done considerable chigger collecting it seems that the "white dish" method is the most satisfactory known at the present time for collecting large numbers of chiggers in a short interval. By the use of this method I have collected 105 larval mites in four minutes and 45 seconds. To be sure, this was an exception, but under favorable conditions the average rate of collecting, during the chigger season, approached 550 an hour. As many as 47 chiggers were found on one saucer after one minute's exposure.<sup>2</sup> About 10,000 were collected during the summer from one area of approximately forty square feet, under one group of blackberry bushes.

#### OBSERVATIONS ON THE BIONOMICS OF THE CHIGGER

##### *The food of the chigger*

Although it has long been known that chiggers do not burrow into the flesh, but attach themselves to their host in a manner similar to ticks, this biological fact has for some reason not been appreciated even today by many trained biologists. There appears to be even less comprehension of the true nature of the food of the chigger. It long has been, and still is, taken for granted by all too many that chiggers feed on the blood of the host which they attack. However, evidence to the contrary began to appear as early as 1871 when Gudden found a tube, which he thought to be a structure of the mite, extending .3 mm. into the skin from the point of attachment of the mites. In 1876 Flögel studied spiders parasitized by larval mites and observed rather long hollow tubes extending into the exoskeleton where the chiggers were attached. Jourdain (1896, 1899) found similar structures on certain Hemiptera and Neuroptera as well as on field mice. Later workers found changes in the chitin of grasshoppers, *Dytiscus* beetles and mosquitoes caused by the attacks of "red-bugs" and realized that these tubular structures were not part of the mite but rather the effect of the bite

<sup>2</sup> Ten-inch white plates were used while collecting chiggers in Florida during the summer of 1945. It was not unusual to collect 150 or more from one plate after one minute's contact with the ground. Two thousand mites were collected in two hours.

upon the host. Talice (1929) points out that the food of larval mites parasitizing animals is probably not blood. In the same year Vitzthum wrote, "... they live on body juices of the host without ingesting blood." Fuss and Hauser (1933) found that the food of chiggers consists of particles of tissues which have been extraorally digested by the secretions of the mites and then drawn up into their gut. It is possible that blood may be ingested by the chigger but this is not true in most instances and probably never does it serve as the chief source of food. A good discussion of the history of this subject is given by Feng and Hoeppli (1933).

A study by various workers with histological preparations of bites have shown the formation of a tube-like structure extending more or less deeply into the skin from the mouthparts of the mite larvae. This tube in mammals, including man,



FIG. 1. Histological Preparation of a Chigger Bite in a Rabbit Ear

Section of a bite showing the tube lined with stratum germinativum cells and containing partially digested exudate of tissue fluids and cells.

has been found to be comparatively short, without branches and lined by a distinct layer of cells. In parasitized mosquitoes this tube is fairly long and without branches, but in spiders, Hemiptera and Neuroptera there are ramifications. Although it is possible that the species of mite may influence the reactions on the part of the host it appears from a comparison that the histopathological changes depend primarily on the kind of host.

The histological preparations of chigger bites on rabbit ears show (Fig. 1) that the epidermis is completely penetrated. A tube lined with stratum germinativum cells is formed which extends to the derma and subcutis. This tube appears to represent a reaction of the host to the secretion of the parasite and its inner layer of cells are necrotic and give evidence of digestion. There is a noticeable degree of round cell infiltration in the area of the tube-like structure. After removal of the chigger the tube contains a partially digested exudate of tissue fluids and cells. The chief source of food would appear to be derived from

an infiltration of cells and fluid which are "sucked" through the inner end of the tube formed by the protective wall of stratum germinativum (Fig. 2). The protective wall grows and eventually closes off the opening at the inner end of the tube.



FIG. 2. Histological Preparation of a Chigger Bite in a Rabbit Ear  
An infiltration of cells and fluid being "sucked" through the inner end of the tube.



FIG. 3. Histological Preparation of a Chigger Bite in a Rabbit Ear  
The protective wall closes off the opening at the inner end of the tube which it forms and when dried the homogeneous zone may drop or be pulled out as a whole.

tube which it forms (Fig. 3). As the liquefied center or homogeneous zone dries and contracts it may drop or be pulled out as a whole from the bite (Fig. 3).

#### *Overwintering larvae*

It is generally believed by biologists that the adult form is the only overwintering stage. This is an erroneous assumption. Japanese workers have observed voles throughout the year carrying the larvae of *Trombicula akamushi*

in their ear lobes. Gladys Keay (1937) found *T. autumnalis* in the ears of rabbits and bank voles during the winter months in England. On December 10 I examined a wild rabbit which had been killed the day before. Three chiggers were found attached to the eyelids and many were found deep in the ears nearly to the membrane. The inner tissue of the ears in the locality of the "red-bugs" was so soft, loose, and fleshy that the chiggers could easily dislodge themselves in a few seconds and could readily be picked out with forceps when attached. All of the larvae were fully engorged, some attached, others unattached and wandering about. It may be possible that the soft condition of the flesh was caused by the secretions of the mites which continued to act upon the cells after the larvae had become detached. It would seem that a dozen or so of these parasites could cause this condition over a period of weeks when they feed first in one place then in another. Periodic feeding seemed to be evidenced by chiggers wandering about unattached and the presence of numerous piles of white excrement, which far outnumbered the chiggers, throughout the region where the mite larvae were found.

On January 16, the head of a rabbit which had been killed on the fourteenth was examined. This head had been kept in a refrigerator prior to examination. One living chigger was found in each of the ears and four others, which proved to be dead upon microscopic examination, were completely embedded in the jelly-like flesh. It may quite possibly be that these four burrowed into the soft flesh when put into the refrigerator, in an attempt to escape from the excessive cold. The quantity of jelly-like tissue and the numerous piles of excrement would seem to indicate that there had been many more present in the not-too-distant past. The absence of sufficient heat may have caused them to migrate in a vain attempt to locate warmer surroundings.

The heads of two squirrels, which had been killed at the same time as the last mentioned rabbit and which had received the same treatment, were also examined. One alive chigger was found in each of the four ears, but again there was evidence that many others had been there recently. There were several mammalations or papule-like structures on the soft, fleshy tissue. When pressure was applied to these papules, causing them to erupt, strands of a white material would burst forth. These strands may well have been degenerate cells left in the homogeneous zone formed by the feeding of the chiggers.

Three wild mice were examined, one on January 18 and two on January 19. No chiggers were found and there was no evidence that any had been in the ears recently. More mice should be examined although it may be possible that the chigger is more specific in its selection of winter hosts than of summer hosts.

#### *Evidence of a negative geotropism*

Very frequently the larval mites would be seen running along the edge of a collecting saucer. When they were in this location it was rather difficult to collect them with the aspirator. However, when the saucer was held in a vertical position with a chigger at the lowest point, the hexapod larva would immediately turn and scamper upwards towards the center of the dish thus

making its capture easier. Further evidence of this negative geotropism was displayed when the collecting aspirator was held upside down. The "red-bugs" which had congregated between the rubber stopper and the glass wall of the vial could be seen going *en masse* up the sides of the container. After this had been done several times in succession, however, the larval mites no longer followed any definite path but wandered about both up and down as if this tropism had been decidedly reduced or lost entirely.

### *Rapidity of movement*

Very few people have any conception of the rapidity with which these little pests can get about. At a temperature of 30.3°C. these larval mites can run, on the average, a distance of slightly better than one foot per minute. This does not seem so striking until careful thought is given to the size of the creatures under consideration. If man could run as fast for his size as the lowly chigger how fast could he run? By means of a series of experiments the answer to this question was determined. A man would have to run with an average speed of slightly better than 173 miles per hour for four consecutive hours before falling from exhaustion. This seems almost incredible and yet there are among nature's creatures others that are far faster for their size than our common chigger.

### *Predators of chiggers*

Several adult mites belonging to two genera of the family known as "snout mites" or Bdellidae were collected along with the chiggers from the vicinity of blackberry bushes. Since this family of mites is noted for its predaceous habits of feeding on most any small creature it can find, it was suspected that the chigger was no exception. Members of the genera *Bdella* and *Cunaxa*, both of which are well adapted for capturing prey, were collected in the same aspirator with some chiggers. The former was seen to feed in a most frenzied and interesting manner upon a dozen or more of the *Eutrombicula* larvae. The "snout mites" are quick in motion and move backwards as well, and as easily, as forwards. Although Banks (1915) implies that these mites do not hold their prey with their long palpi it looked as if they made a good attempt at doing so. If a chigger happened to brush against any portion of the *Bdella* the "snout mite" would whirl and grasp the chigger with palpi and forelegs and with rapid hammer-like strokes pierce it three or four times with its long beak-like snout. After treating some four or five "red-bugs" in this manner it would return to the kicking prostrate forms of the chiggers and more leisurely suck the juices from within their bodies, leaving nothing but the shriveled exoskeleton.

### SUMMARY

1. A new and rapid method of collecting chiggers in nature is described.
2. Rarely do chiggers feed on blood and never is it their chief source of food. An extraoral digestion of epidermal cells is practiced by this parasite.
3. Chiggers overwinter in the larval stage deep in the ears and on the eyelids of rabbits, squirrels, and possibly other animals.

4. "Red-bugs" are negatively geotropic and possess amazing speed for their size.

5. Chiggers are frequently preyed upon by members of the mite family Bdellidae.

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## A METHOD OF REARING CHIGGER MITES (ACARINA, TROMBICULINAE)<sup>1</sup>

CHARLES D. MICHENER<sup>2</sup>

Although chiggers are severe pests in many regions of the world and are important vectors of disease in a large area of the Far East, satisfactory rearing methods for these mites have not been found. Rearing is peculiarly important for disease transmission studies with chiggers, since an individual mite feeds on a vertebrate host but once during its entire life and in but one stage, the larva. The disease organisms must, therefore, pass from the larva of one generation through all successive stages, including the egg, to the larva of the next generation. While there has not yet been achieved the goal of carrying through the cycle from larva to larva without serious mortality, the method described below may serve as a basis for the development of a satisfactory technique. In its present form, however, the method has been of great value in studying all stages of the life cycle and in obtaining the taxonomically important nymphs and adults from larval chiggers.

The studies upon which this paper is based were carried on with a common species which infests man in Panama, *Eutrombicula batatas* (Linnaeus).<sup>3</sup>

Evidence now being accumulated indicates that certain other species, for example, *Trombicula velascoi* Boshell and Kerr, may be reared in the same manner.

Since domestic chickens are an important and convenient host of *E. batatas*, they have been used as hosts for the chigger larvae in most of these studies. Fully engorged larvae can be obtained in large numbers and in good condition by placing a naturally infested chicken in a cage, the bottom of which is made of coarse (one-half inch) wire mesh. The cage is provided with short legs and is placed in a large tray filled with water (Fig. 2). As the engorged larvae fall from the chicken they pass through the wire mesh and land on the water, floating helplessly on the surface. It is desirable to have the water at least one inch deep so that the feces of the chicken will sink below the surface. The water in the tray is changed after each collection of the chigger larvae. Food and water for the chicken are provided in very small containers in order to interrupt the fall of as few mites as possible, and rice or white bread is used as food so that scattered particles floating on the water will not seriously interfere with finding the red larval chiggers.

<sup>1</sup>The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Gorgas Memorial Laboratory, Panama City, R de P.

The author is much indebted to Dr. Herbert C. Clark, Director of the Gorgas Memorial Laboratory, for excellent facilities provided for this work, and to Major Marshall Hertig, Sn.C., and Captain G. B. Fairchild, Sn.C., for assistance and helpful suggestions.

<sup>2</sup>Captain, Sn.C., A.U.S.

<sup>3</sup>The use of this name is discussed in a forthcoming paper. It is the same species which has sometimes been called *Acariscus hominis* (Ewing).

Twice a day until the chicken is free of chiggers (a week to 10 days) the engorged larvae are collected from the water surface onto small squares of paper. The corner of a piece of paper is dipped into the water beside a floating chigger

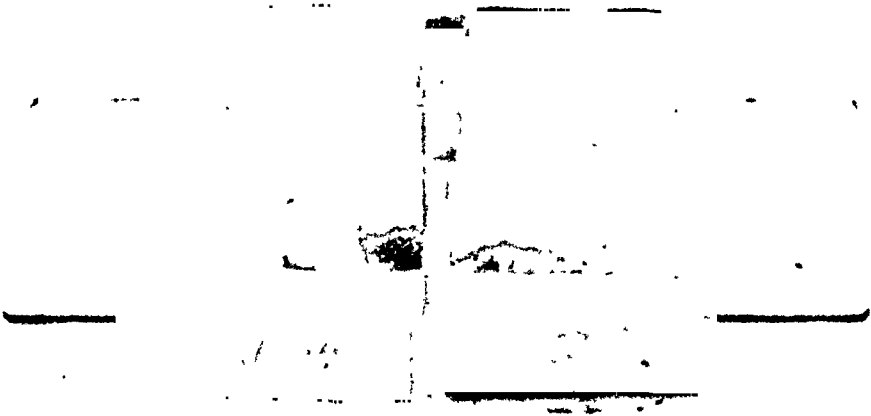


FIG. 1 Plaster of Paris cell covered with microscope slide, for making observations on eggs and other stages of chiggers. (Photographs, courtesy of U. S. Army Signal Corps.)

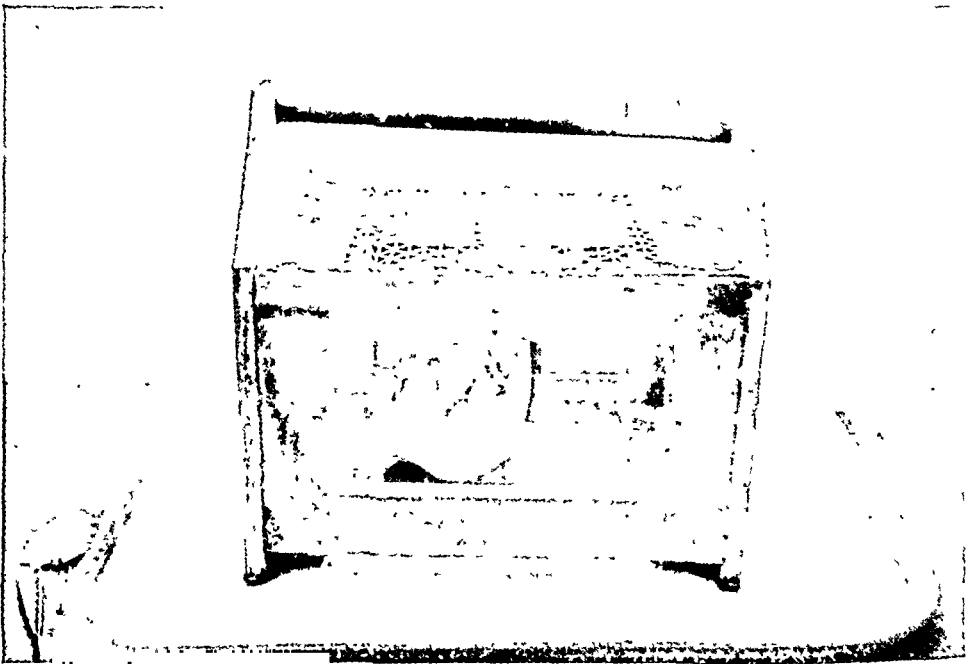


FIG. 2. Animal naturally infested with chiggers in cage standing in tray of water. Engorged larvae fall to water where they float and may be removed by means of squares of newspapers.

and on being withdrawn from the water the surface tension causes the chigger to stick to the paper. Newspaper has the proper absorptive qualities for this purpose. As the squares of paper dry the mites are released and walk about.



The moist squares of paper with the mites on them are placed in half-pint fruit jars lined on the bottom and sides with a layer of plaster of Paris one-eighth to one-fourth inch in thickness (Fig. 3). Such jars are prepared by pouring into them a rather viscous mixture of plaster of Paris and water and then turning and tilting the jars slowly until the sides and bottom are covered with a layer of uniform thickness. Continued turning is sometimes necessary until the plaster solidifies. This layer of plaster of Paris is of great importance, as it absorbs water which would otherwise condense on the glass. In unlined jars most of the engorged larvae become stuck in such water and die without trans-

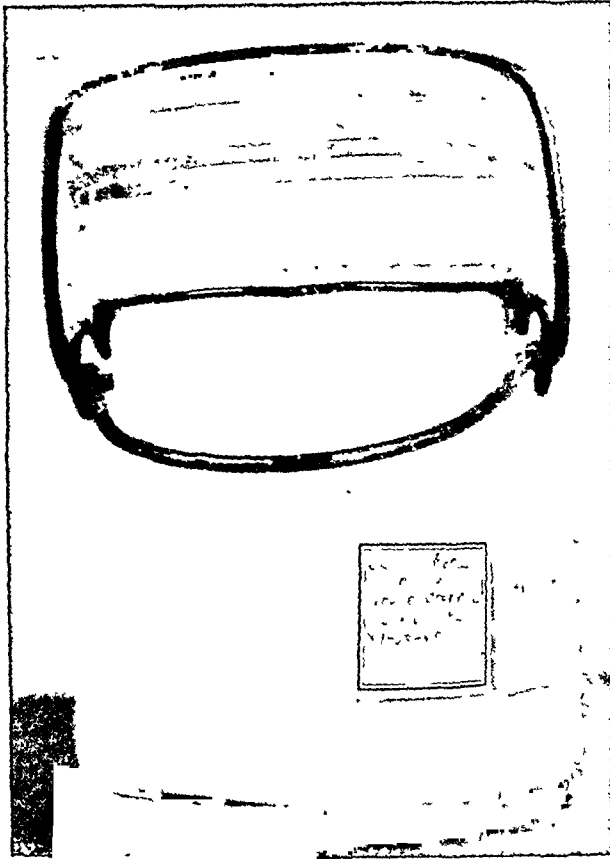


FIG. 3. Fruit jar with inner coating of plaster of Paris, for rearing engorged larvae to nymphal stage.

forming. Since engorged larvae appear to seek darkness, no difficulty is encountered with larvae crawling onto the unlined glass jar lids. The use of opaque metal lids is objectionable, however, as the larvae commonly crawl onto the lids. In order to maintain a high humidity, a few drops of water are placed in these jars whenever the paper or plaster appears dry.

It is frequently important to obtain engorged larvae for rearing from wild animals or birds which cannot practicably be caged over a pan of water. This may be done by placing the dead body of an infested animal in a paper sack which is sealed with paste. After 24 hours the sack is opened and the chiggers which have left the animal are shaken into a plaster-lined jar. Others are removed by

clipping pieces of skin to which they are attached from the body of the host and placing these pieces in the jar. The latter method alone is frequently satisfactory. Of course only the fully engorged larvae survive.

Within a week or 10 days engorged larvae transform to nymphs in the plaster lined jars with very little mortality. (Longer periods are required for some species.) When all have transformed the nymphs are shaken out of the jars lined with plaster of Paris into prepared rearing jars. The most successful rearing jar so far used is a pint fruit jar with the bottom broken out and replaced by a plug of plaster of Paris about three-fourths of an inch thick (Fig. 4).

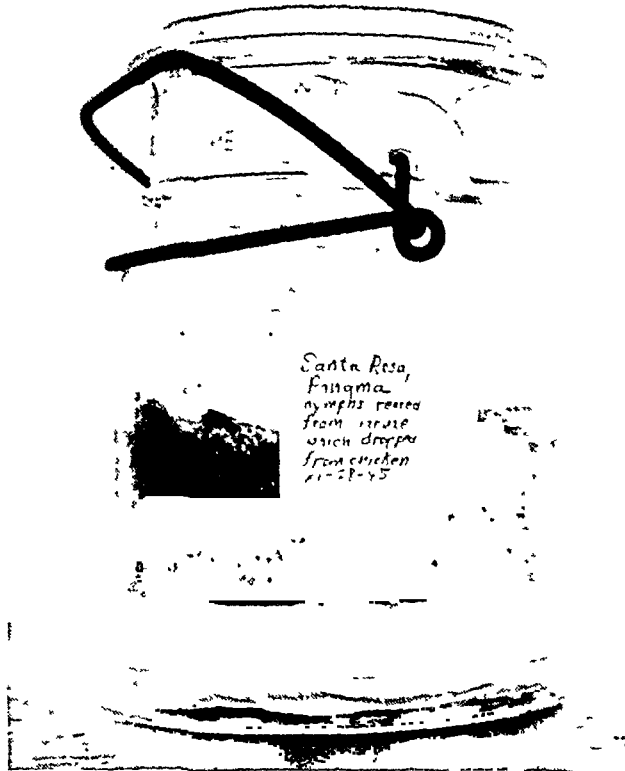


FIG. 4. Rearing jar made by replacing bottom of fruit jar with plaster of Paris. Nymphal and adult chiggers live in mixture of soil and chicken manure.

A container made by plugging one end of a lamp chimney with plaster of Paris is equally satisfactory except that the top is more difficult to seal. In the jar is placed a mixture of about 5 parts moist, sterilized soil to 1 of chicken manure, as suggested by Melvin.<sup>4</sup> This mixture is then tamped down gently to form a layer about  $1\frac{1}{2}$  inches thick and the jar is ready for the nymphal chiggers. Various procedures, such as addition of growing green plants, small soil insects, sterilization to reduce mould and bacterial growth, etc., have had little effect on the growth or mortality of the nymphs.

<sup>4</sup>Melvin, Roy. Note on the culturing of chiggers. (In press.)

Best results are obtained when the top of the jar is left open. Nymphs and adults stay on or in the moist soil and do not climb out of the jars if they are open (and therefore relatively dry above the soil level). Water is added daily. Any excess passes through the plaster plug in the bottom of the jar into a petri dish in which the jar stands. Since there is a gradient in the soil from relatively dry above to very moist below, the mites are able to take up positions of favorable humidity.

Transformation to adults takes place in the soil three or more weeks after the nymphs are placed in the rearing jars.

Similar rearing jars are used for wild-caught nymphs and adults. When eggs are desired for study it is advisable to tamp the soil very firmly so that the adults cannot get beneath the surface. Then the eggs will be found on the surface. If only larvae are desired this is unimportant, since they will crawl up out of the soil upon hatching. When larvae are expected to appear in a jar the lid is clamped on with a rubber seal so they cannot escape. The petri dishes in which the jars stand are placed on squares of paper saturated with dimethylphthalate in order to kill any larvae which escape when the lids are removed for addition of water, removal of larvae, etc.

Larvae to be placed on a host animal are removed from the rearing jar by picking them up on small pieces of wet newspaper. The paper is then either fastened to a feather on the under surface of the wing of a young chicken in such position that the larvae will crawl off onto bare skin (surrounding feathers plucked if necessary) or the paper with its mites is placed in a very shallow, wide, shell vial, the open end of which is pressed closely to the skin of the host and held in position with adhesive tape. In either case as the pieces of paper dry the mites are released from the water film, and after wandering about, attach themselves to the host. Since unengorged larvae can walk on the surface of water and are not easily held by a water film, the entire operation must be performed rapidly. If the mites are released on the dense feathers of well-grown chickens a high proportion drop off without working through the feathers to the skin.

After engorgement mites are retrieved from the host in the same manner as described above for obtaining engorged larvae from naturally infested chickens. Some drop within 2 days after attachment, while others remain attached for as much as 10 days.

A small plaster of Paris cell covered with a microscope slide (held in place with a rubber band) is convenient for making special observations, for example, on eggs and later quiescent stages (Fig. 1). Such cells have the advantage that water can be added from the outside through the plaster without disturbing the contents. Nymphs and adults, however, do not appear to survive for more than a week or two in constant contact with plaster of Paris.

Using the methods here described, wild-caught females have been induced to lay eggs and from these eggs a first generation of adults has been reared. However, the mortality has been high, especially in the nymphal stage, and of the larvae hatched only about 10 percent finally yielded adults. Engorged wild-caught larvae from chickens have been reared through to adults but here again

the mortality is about 90 percent, mostly in the nymphal stage. Adults reared from wild-caught engorged larvae have in a few cases laid fertile eggs which yielded larvae, but it was not possible to rear these few larvae to adults. It is possible that the difficulty encountered in securing eggs from laboratory reared adults may be due to the disinclination of adults to mate in captivity.





DR. CHARLES MORLEY WENYON

1945 RECIPIENT OF THE THEOBALD SMITH GOLD MEDAL OF THE GEORGE WASHINGTON  
UNIVERSITY, AWARDED BY THE AMERICAN ACADEMY OF TROPICAL MEDICINE

REMARKS MADE BY THE AMERICAN AMBASSADOR ON  
PRESENTING THE THEOBALD SMITH GOLD MEDAL  
To DR. CHARLES MORLEY WENYON

AT A MEETING OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND  
HYGIENE AT MANSION HOUSE, PORTLAND PLACE, LONDON

On January 17th, 1946

I am happy to come here this evening at the request of the officers of the American Academy of Tropical Medicine to assist in doing honor to your distinguished president, Dr. Charles Morley Wenyon.

Today we believe that the men of all nations must work together for the common good. Our governments and our peoples are giving deep thought and earnest effort to establishing channels of communication through which men may learn to understand other cultures and so find the common denominators of purpose. They are endeavouring to set up machinery through which economic, political and social cooperation may be established. We have faith in the ultimate success of these efforts, though the way is difficult. We find new encouragement when we turn to the world of science where cooperation has long been a living fact and great men work selflessly in the pursuit of truth for the benefit of mankind. During this war British and American scientists have worked together more closely than ever before; joint efforts have given to the world the benefits of penicillin, DDT and radar.

I wish to speak of Dr. Wenyon not only as an eminent man of science, an authority on tropical disease, but also as a world citizen. During this global war, soldier and layman have become increasingly aware of his contribution in life-saving. Even greater than the hazards of high explosives were the dangers of disease in desert and jungle. Yet the casualties from these causes have been lower than we dared to hope. We are beginning to understand something of what the advances in knowledge of tropical disease mean to millions of people living in infected areas. Dr. Wenyon has played a great part in this through his own investigations of protozoan diseases and of malaria and by the leadership and guidance which he has given to others in this field.

Dr. Wenyon on behalf of the American Academy of Tropical Medicine, I present to you the Theobald Smith Gold Medal. As a student you so distinguished yourself at the University of Leeds, Guy's Hospital and the Pasteur Institute that you were called by that great pioneer in tropical medicine, Sir Patrick Manson, to be the first protozoologist at the London School of Hygiene and Tropical Medicine. You later became Director of the Bureau of Scientific Research of the Wellcome Research Institution and then Director in Chief of that Institution. You now carry on as an honoured consultant and leader. Your laboratory has been in many lands: the Sudan, Malta, Mesopotamia and Greece. As one surveys your career one feels a strong element of the romance of exploration as well

as of tireless labour. Your name and work are known not only to those who are privileged to call you friend, but also to countless numbers you have benefited.

I understand that you are already an honorary member of the American Academy of Tropical Medicine. You now become the first man outside America to receive the Theobald Smith Gold Medal. Your American colleagues take great satisfaction in this award. They only regret that it has not been possible for you to visit them personally to receive it.

I take great pleasure in reading the citation and in handing to you a medal which is a symbol of the esteem in which you are held by the scientists of the world.

#### THE CITATION

On behalf of the American Academy of Tropical Medicine this Theobald Smith Gold Medal is conferred on

#### CHARLES MORLEY WENYON

Pioneer in tropical medicine, he has brought light when there was darkness; not hesitating to experiment on himself he has obtained direct answers to many important questions in disease transmission and through his discoveries he has made the world a better place to live in for all peoples without regard to race, creed, color or economic status; he is one of those leaders who are to the everlasting credit of the British Empire.

#### *Dr. E. M. Wenyon's Reply:*

Your Excellency:

I must thank you for the very kind words you have spoken, and would ask you to convey to the President and Council of the American Academy of Tropical Medicine my appreciation of the great honour they have done me in awarding me the Theobald Smith Gold Medal with which, on their behalf, you have just now so graciously presented me.

I am all the more gratified by the award when I reflect that Theobald Smith made one of the most important discoveries in protozoology—the only field of science in which perhaps I can lay any claim to distinction.

It was over half a century ago, in 1889 in fact, that Theobald Smith described a protozoan organism, now known as *Babesia bigemina*, which he had discovered in the blood of cattle suffering from Texas fever, or red water fever, as it is more generally termed. This, in those days, was a remarkable enough observation but, not content to rest on his laurels, he set to work with Kilborne on a long series of laborious investigations into the method of spread of the infection, then an unsolved problem which appeared to be connected in some way with the soil. These were crowned with success, for four years after the announcement of the discovery of the organism he had proved not only that it was transmitted by the cattle tick *Boophilus annulatus* but that it passed from one generation of tick to the next through the egg. This is necessarily so, for the tick is a one host tick



which, having gained a footing on an animal in the larval stage, remains on the skin for the rest of its life, feeding on blood till the fertilized female finally falls off to lay her eggs on the ground before dying. In due course larvae hatching from the eggs crawl up blades of grass and quickly infest any cattle which come into contact with them. If the host from which the female tick dropped in the first place harboured the parasite in its blood, then the female tick itself was infected and passed the infection by way of its eggs to all the larval ticks which hatched from them. Such infected larvae never failed to infect any susceptible cattle from which they took their first feed of blood. All this and much more was described in detail by Theobald Smith and Kilborne in the first Bulletin issued in 1893 by the U. S. Department of Agriculture, a publication which is a classic and a model of what such a report should be. It is one which should be read by everyone seriously interested in the arthropod transmission of disease.

This indeed was a stupendous discovery, the first demonstration that a blood-sucking arthropod could transmit an infection by its bite. It was the fore-runner of a series of similar discoveries made by workers in our two countries in which blood-sucking arthropods were incriminated as vectors of a number of diseases—malaria and yellow fever by mosquitoes, sleeping sickness by tse-tse flies, plague by fleas, and various forms of typhus and relapsing fever by lice and ticks—to mention only a few.

When the American Academy of Tropical Medicine was founded in 1934 it was most appropriate that Theobald Smith should be elected its first President, in recognition of his epoch-making discovery in Protozoology—a subject which is of such great importance in Tropical Medicine. The Council of the Academy then paid me the great compliment of electing me one of its first three Honorary Members.

Theobald Smith died a few months after his election as President, and the Gold Medal with which you, Sir, have this evening presented me was founded in his honour. This is the fifth award of the medal, the previous recipients being Barber, Strong, Stitt and Craig—names which are too well known throughout the world in the field of Tropical Medicine to need any comments from me. They are all Americans, and I may perhaps be excused if I take some pride in the fact that I am the first representative of another nation, albeit a very closely related one, to receive this high award. I accept the medal at your hands, Sir, in all humility, realizing that the honour is not for me alone but is a recognition by the American Academy of the important part Great Britain and its Empire have played in the development of tropical medicine.

During the war our two nations have been thrown very close together in many tropical centres of activity. In the field we have been jointly occupied in the solution of many tropical medical problems, while knowledge acquired by investigations carried on in our respective countries has been fully shared to our mutual advantage. Now that the war is over it is to be hoped that this close association will be continued and that the many new contacts and relationships which the war has brought about will not be broken. It appears to me that this award by the American Academy of Tropical Medicine sets a seal to the continu-

ance of this mutual respect and understanding which have long existed between investigators in our two countries in the field of tropical medicine.

I must again thank you, Sir, not only for honouring our Society by coming here this evening but also for giving me the very great satisfaction of receiving at your hands the Theobald Smith Gold Medal of the Academy of Tropical Medicine of the great country which you represent—a country which has made and is still making such valuable contributions to knowledge in this branch of medicine. Your country and mine have very great responsibilities, for the health and well-being of the millions who are destined to pass their lives in tropical lands, where they are beset by many and often mysterious dangers, depend to a large extent on our knowledge of the diseases of these areas and, above all, on the successful application of this knowledge by the men and women we train to put it into practice. In this respect our purpose is one, which may very suitably be expressed by the motto of this Society—the Royal Society of Tropical Medicine and Hygiene—*Zonae torridae tutamen*.

# ISOLATION OF YELLOW FEVER VIRUS FROM AFRICAN MOSQUITOES

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When the Yellow Fever Research Institute was opened at Entebbe late in 1936, general epidemiological investigations were undertaken to ascertain the scope and intensity of activity of the yellow fever virus in East Africa, particularly in the southern Sudan and Uganda. Immunity surveys reported by Sawyer and Whitman (1) had already shown that yellow fever had occurred there, so that our earliest studies were directed toward extension of their findings and the search for suitable localities for intensified epidemiological investigations. When, in 1937, Dr. J. C. St. G. Earl, then Senior Medical Officer in the Western Province of Uganda, collected a series of blood specimens from African adults in Bwamba County and a high incidence of immunity was found among them (2), this area was decided upon for more intensive studies. Investigations have been continued in Bwamba since that time, with short interruptions necessitated by the war.

Bwamba is a small county, covering an area of about 140 square miles, between the Ruwenzori Mountains and the Semliki River, in the extreme west of Uganda. About 80 square miles of the county is covered with uninhabited dense primeval rain forest, but the remainder is fairly intensively cultivated and supports a population of over 30,000 persons. The topography and vegetation have been described in detail elsewhere (3).

Before proceeding further it should be mentioned that in Bwamba the behavior of the classical vector of human yellow fever, (*Aedes (Stegomyia) aegypti* L.), is of an unusual nature (3). It occurs in certain uninhabited forest areas, where the larvae may be taken in fair numbers in tree holes but adults can only occasionally be taken biting man. *A. aegypti* is not abundant in any part of Bwamba, and it is definitely scarce in the inhabited areas and in huts. All the evidence at present available indicates that it probably has no important relationship to the local epidemiology of yellow fever.

An outbreak of yellow fever occurred in Bwamba County in 1941 (4), during the course of which yellow fever virus was isolated from the blood of a sick African and from two lots of *Aedes (Stegomyia) simpsoni* Theobald. Mass immunization of the human population was carried out at that time with excellent results (5), so that it was not to be expected that yellow fever could recur there in epidemic form in the near future. Comprehensive immunity surveys (2, 6) however, had already shown that by far the highest incidence of immunity in humans occurred in the areas adjoining the edge of the main forest, which is

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known as the Semliki Forest and which itself is entirely uninhabited. Immunity in children was almost restricted to these areas. Further, subsequent surveys of immunity among forest monkeys (7) revealed a high percentage of immunes, all the known lowland species being involved. The immunity rate among the first 150 monkeys obtained in Bwamba was 61 per cent.

As early as 1942, entomological investigations directed toward the further study of *A. simpsoni* as a vector and the discovery of other possible insect vectors were centred in the areas along the edges of the Semliki Forest, still in rather close proximity to human habitations. As will be shown, yellow fever virus was isolated from *A. simpsoni* caught in a forest-edge locality in June 1942, 11 months after the human population had been immunized, indicating strongly the existence of an extrahuman cycle of activity. It seemed most likely that this cycle must involve the animals of the Semliki Forest and that, as *A. simpsoni* enters only the extreme edges of this forest, some other insect vector must be involved. Consequently, attempts to recover virus from insect vectors were thereafter confined to mosquitoes collected in uninhabited areas of the primeval forest. Yellow fever virus was isolated from a mixed lot of *Aedes* spp. (including neither *A. aegypti* nor *A. simpsoni*) collected in uninhabited rain forest in April 1944. This was the first time that the virus was ever isolated from forest mosquitoes in Africa, and it gave the final proof of the existence of the disease independent of the human host on that continent.<sup>3</sup>

#### METHODS

*Mosquito catches.*—In the case of the earlier catches, large numbers of humans who remained stationary were used as bait, a few trained catchers picking off the mosquitoes as they arrived to bite. Later, particularly when working in the more remote forest areas, it was found more convenient to use a squad of about 20 experienced catchers, who moved singly through the forest, collecting the mosquitoes which alighted to bite them. In this way a large forest area could be covered rapidly, the squad of 20 obtaining as many as 400 mosquitoes per hour. Mosquitoes seen resting on tree trunks and in the undergrowth were also collected but were kept separate from those which had been taken biting. Catches of over 1,000 mosquitoes per day have been quite common, and on several occasions over 2,000 have been taken in a single day.

All mosquitoes were caught singly in short test tubes, which were then lightly plugged with cotton. At the field laboratory the specimens were sorted and identified without removing them from the tubes. After classification, they were released in Barraud cages and were fed on banana and water until such time as they were sent to the Institute at Entebbe for inoculation into laboratory

<sup>3</sup> We herein avoid the use of the term "jungle yellow fever" because that term is defined (8, 9) as "yellow fever in the absence of *Aedes aegypti*." This insect is not absent in the area where our studies were made, and this strict definition therefore does not cover the local situation. However, as already stated, we do not believe that *Aedes aegypti* plays any important rôle in the epidemiology of yellow fever in Bwamba, so that the extrahuman cycle to which we refer is, in many if not all respects, comparable to the jungle yellow fever of South America.

animals. These methods, which reduce the handling of the insects to a minimum, have been described more fully in a separate communication (10). As a rule the catches were made in series of five, on consecutive days.

On account of the extremely prolific and varied nature of the Bwamba mosquito fauna, it was not possible to inoculate each species separately. In most cases a generic grouping was used, though quite frequently particularly abundant or suspect species were isolated for separate inoculation.

For transportation over the 275-mile journey by road to the laboratory, the Barraud cages were placed in carrying cases provided with close-fitting covers and screened windows for ventilation. A pad of moist cotton was placed over each cage to prevent desiccation. Deaths of insects in transit were not excessive.

*Inoculations.*—Mosquitoes were inspected in their cages on arrival at the laboratory in Entebbe. In some instances the dead were discarded while in others they were used together with those which were living. The viable insects were killed with chloroform and, with or without those which were dead on arrival, were ground in a sterile porcelain mortar. In the case of large lots of insects, sterile powdered pyrex glass was used as abrasive to facilitate suspension. After thorough maceration the insects were suspended in the minimum quantity of 10 per cent serum<sup>4</sup> required to make a good suspension. This was spun in the angle centrifuge for 20 minutes to one hour at about 3,000 r.p.m., and the supernate was removed. Usually a 1 ml. portion of the supernate was passed through a washed Seitz EK pad in preparation for intracerebral inoculation of mice,<sup>5</sup> but the remainder of the supernate was not filtered. On occasion unfiltered supernate was also inoculated into mice, but usually the entire unfiltered portion of supernate plus any remaining portion of filtrate was inoculated subcutaneously into a normal rhesus monkey. The number of lots of mosquitoes and the numbers of individual insects ground up for inoculation into animals during the three-year period of the study are shown in table 1, together with the number of monkeys and groups of mice receiving the suspensions.

*Observations of Animals.*—Mice were examined daily in the forenoon, and brain passages were made from any which appeared ill. Monkeys were observed at frequent intervals and their temperatures were taken twice daily, except on Sundays and holidays, when they were taken in the morning only. Whenever a monkey had fever of 104°F. or higher, or appeared ill, it was bled from a leg vein, and subinoculations<sup>1</sup> were made to mice and occasionally to another normal monkey. Monkeys which succumbed were examined post mortem, and stained sections of their tissues were studied microscopically. Protection tests were done on all monkeys prior to the first inoculations. These were repeated before each reinoculation if the monkeys were subsequently used. The final tests for immunity were made at least one month after the last inoculations. The strains of virus isolated were identified by protection tests in mice.

<sup>4</sup> Ten per cent serum is routinely used in this laboratory as diluent for yellow fever virus or any materials suspected of containing that agent. It is prepared by adding 9 volumes of sterile physiological saline to one volume of known non-immune serum. It is routinely passed through a Seitz EK pad prior to use.

<sup>5</sup> For this and other experimental procedures animals were anesthetized with ether.

ISOLATION OF VIRUS FROM *AËDES SIMPSONI*

Intensive collection of mosquitoes was begun in Bwamba in March 1942. At first, attention was directed almost entirely to the collection of *A. simpsoni* in banana plantations, where this axil-breeding species abounds. During the period from March 6 to June 14, 2,276 mosquitoes were obtained, 2,207 being *A. simpsoni*. Of these, 1,926 were sent to Entebbe in five lots. Lot No. 5, consisting of 432 *Aedes simpsoni* females caught at Bundinyama, arrived at the laboratory on the evening of June 15, 1942. The next morning 37 of the mosquitoes were dead, and these were discarded. The 395 viable insects were killed with chloroform and ground in a mortar with 7.9 ml. of 10 per cent serum saline. The suspension was cleared by centrifugation, and six mice were inoculated intracerebrally with the unfiltered supernate. The remainder of the supernate was passed through a

TABLE 1

Summary of inoculations of animals with suspensions of wild-caught mosquitoes over the three-year period 1942-1944

YEAR	MOSQUITOES: NO. OF LOTS	NO. MOSQUITOES USED FOR INJECTION	NO. INOCULATIONS DONE	
			Groups of mice	Rhesus monkeys
1942*	5	1,781	8	5
1942††	33	17,468	33	33
1943§	43	31,042	43	43
1944  ¶	31	16,417	31	27
1944**††	125	18,131	371	94
Total.....	237	84,839	486	202

\* Up to and including the isolation of yellow fever virus from *A. simpsoni*.

† After isolation of yellow fever virus from *A. simpsoni*.

‡ Semliki Forest virus (11) isolated.

§ Bunyamwera virus (12) and another undescribed virus isolated.

|| Up to and including the isolation of yellow fever virus from mixed *Aedes* spp.

¶ Two strains of Rift Valley fever virus isolated (18).

\*\* After the isolation of yellow fever virus from mixed *Aedes* spp.

†† Four strains of Rift Valley fever virus isolated (18).

Seitz EK pad. Another group of mice received filtrate intracerebrally, and the remaining 6.0 ml. of filtrate was inoculated subcutaneously into normal rhesus monkey No. 240.

All the mice which received unfiltered mosquito suspension were dead the day following inoculation, and they were discarded. The six mice which received Seitz-filtered suspension remained well through 30 days and were discarded.

The normal temperature of rhesus 240 was 101.4° to 102.4°F. On the seventh day (June 23) its temperature rose to 103.8°F. and was elevated daily thereafter until June 28, but did not at any time exceed 103.8°. The animal did not appear particularly ill until June 28, when its coat was rough and it seemed weak and apathetic and refused food. The animal was found dead on the morning of June 29. The gross autopsy was quite suggestive, and microscopic sections revealed the presence of lesions of yellow fever.

Rhesus 240 was bled on June 28, when the first sign of illness appeared. The serum obtained was subinoculated intracerebrally into a group of mice, and rhesus 244 received 1.0 ml. intraperitoneally. The mice sickened on the fourth day, and all were dead by the sixth day. Intracerebral inoculations in series were successful, the virus becoming established readily in mice after passage through the rhesus monkey, although it failed to become established in mice directly from the mosquitoes.

Rhesus 244 had an afternoon temperature of 104.0°F. 30 hours after inoculation. It first showed outward signs of illness on the morning of the second day after inoculation, but by the afternoon of the third day it was moribund. It was then bled to get serum for preservation by drying, and sacrificed for autopsy. The stomach contained flecks of black altered blood mixed with partially digested food, and the mucosa was congested. The liver was normal in size, pale yellow-brown in color, and greasy in consistency. Stained sections of the tissues exhibited the lesions of yellow fever.

Serum taken from rhesus 244 three days after inoculation was used as virus in an intraperitoneal protection test. To 3.0 ml. portions of normal and yellow fever immune sera were added 1.5 ml. portions of the monkey serum; 0.6 ml. portions of the two mixtures were inoculated intraperitoneally into separate groups of six mice previously prepared by the intracerebral inoculation of sterile 2 per cent starch solution. The mice receiving monkey serum virus mixed with normal serum sickened, and all were dead by the twelfth day, whereas those receiving monkey serum virus mixed with yellow fever immune serum were protected and all remained well.

Bundinyama, the locality where this lot of mosquitoes was caught is situated near the southern border of the Semliki Forest, with which it is connected by a dense strip of relict primary forest. The mosquitoes from which the virus was isolated were caught in a banana and coffee plantation not 100 yards from the edge of this strip. After yellow fever virus had been isolated from these *A. simpsoni*, catching of mosquitoes was started in the relict forest itself, in an attempt to ascertain whether virus could be isolated from other species. Although the catches were continued in this forest belt during two months, yellow fever virus was not again isolated. In the second month, however, another virus, apparently hitherto unknown, was isolated from a lot of mosquitoes belonging to an undescribed species of the *Aedes* (*Aëdimorphus*) *abnormalis* Theo. group and was given the name Semliki Forest virus (11).

As has been explained above, the conclusion was reached that yellow fever must be persisting among wild animals and that some vector other than *A. simpsoni* must be involved. Attention was therefore turned to the main uninhabited forest area, and intensive collection of forest mosquitoes was begun.

#### ISOLATION OF VIRUS FROM FOREST AÆDES SPP.

Between the time of the isolation of virus from *A. simpsoni* in 1942 and the end of 1943, 61,031 mosquitoes were collected in various parts of the Semliki Forest, and of these 48,924 were dispatched to Entebbe for inoculation into laboratory animals. Three other viruses (11, 12, 13), each probably hitherto unknown, were

isolated, but yellow fever was not encountered. We were unable to obtain information from pygmies or other hunters regarding the presence of illness in any forest animals, and there was nothing else to indicate an area in which success might be expected. Whether the disease in forest is endemic or epidemic was not known, and there was certain to be a considerable element of chance associated with success. During an earlier stage of the work it had been thought likely that a comparison between the immunity rates in monkeys shot in different localities might help in the selection of a suitable area, but by this time it had become apparent that the monkey disease was ubiquitous in the lowland forests and that the immunity rate differed but little between one district and another. Such being the case, it seemed rational to concentrate the effort on a single area of uninhabited rain forest, the main requisites in the selection of the area being as follows:

- 1) The continuous presence of a large and varied mosquito population.
- 2) The continuous presence of monkeys in large numbers.
- 3) The evidence of past activity of yellow fever virus, as shown by immunity among monkeys.
- 4) The absence, as far as possible, of human beings.
- 5) Reasonable accessibility by road.

By the end of 1943 a considerable amount of information about the Semliki Forest and its animals had been acquired, and an area known as Mongiro seemed to fulfill the foregoing requirements particularly well. Intensive work was begun there in January 1944.

Mongiro is a small belt of dense primeval rain forest with high closed canopy, lying on the eastern border of the main Semliki Forest. It is continuous with the main forest to the north. To the west it is bounded by an extensive grassy clearing, formerly the site of a thriving village which was abandoned a few years ago by the few who survived a sudden fulminating epidemic. To the east is an area of hot sulphur springs, and beyond these are the open Semliki Plains. To the south the area is bounded by the foothills of the Ruwenzori Mountains on which vegetation of a different kind prevails. The forest at Mongiro is of a very mixed type. The soil is black and rich and is threaded by numerous streamlets which flow all year round. This marshy forest floor supports a dense growth of *Marantaceae* and *Zingiberaceae*, whose large leaves form an excellent harborage for resting mosquitoes. Timber trees of many species occur at Mongiro and the oil-palm (*Elaeis guineensis* Jacq.) is particularly prevalent. The abundance of this palm, which fruits irregularly throughout the year, is a point of importance, as the prolific supply of oil-nuts enables a huge and varied monkey population to thrive in the area. We estimate that there are not less than 400 monkeys in a single square mile of forest at Mongiro. These monkeys belong to nine different species and are divided into 12 bands. Many species of animals belonging to other groups occur at Mongiro. Among the big game, elephant and buffalo are particularly prevalent and serve as a deterrent to would-be native settlers. The area, moreover, is regarded with superstitious dread by the local Africans and, though the main road traverses the forest strip, the entire population within a radius of a mile is less than 10 individuals. So far as can be ascertained all these



have been vaccinated against yellow fever. The nearest huts are about half a mile distant from the forest edge. The general picture is thus of a partly isolated strip of dense lowland rain forest, usually very warm and humid, inhabited by large numbers of monkeys and other wild animals but not by humans, and supporting a huge and varied mosquito fauna. The area has been described in greater detail in a separate communication (14).

Though the classical vector (*A. aegypti*) and three known laboratory vectors of yellow fever (*Eretmapodites chrysogaster* Graham (15), *Taeniorhynchus (Mansonoides) africanus* Theo. (16), and *Aedes (Stegomyia) africanus* Theo. (17)) had been encountered at Mongiro, it was considered possible that some other mosquito might be the forest vector in this area. It was not possible, however, to inoculate each species into a separate monkey, as catches at Mongiro usually included about 30 different species. Generic groupings, therefore, were used for the inoculations, although the composition of each by species was known. As mentioned above, however, particularly abundant species were quite frequently given individual treatment.

During the period between the end of January and mid-April 1944, 14,020 mosquitoes were collected at Mongiro and, of these 13,354 were dispatched to Entebbe for inoculation into laboratory animals. No virus was obtained. Between the 18th and the 23rd of April a catch of 4,310 mosquitoes was made and 3,860 of these were sent to Entebbe, arriving on the evening of April 23. The inoculations were done the following morning. The catch included rather large numbers of the *A. tarsalis* Newst. group and *A. circumluteolus* Newst., which had been kept separate; but all other mosquitoes were grouped generically and were thus inoculated into animals. Separate groups of mice were inoculated intracerebrally with Seitz filtrate of suspensions of *Anopheles*, *Culex*, *Taeniorhynchus*, *Eretmapodites*, *Aedes*, *A. tarsalis* group, and *A. circumluteolus*. Separate normal rhesus monkeys were inoculated subcutaneously with unfiltered supernates of suspensions of *Anopheles*, *Taeniorhynchus*, *Eretmapodites*, *Aedes*, *A. tarsalis* group and *A. circumluteolus*.

This was a most productive catch of mosquitoes, as Rift Valley fever virus was isolated from both the *Eretmapodites* and the *A. tarsalis* group (18) and yellow fever virus from the *Aedes*. However, we are here concerned only with the latter. The lot of *Aedes* from which this strain of yellow fever virus was isolated contained 80 insects of 12 different species, as follows:

<i>A. (Stegomyia) apicoargenteus</i> Theo.....	3
<i>A. (S.) de-bocri</i> ssp <i>de-meilloni</i> Edw.....	12
<i>A. (S.) africanus</i> Theo.....	3
<i>A. (Aedimorphus) haworthi</i> Edw.....	1
<i>A. (A.) argenteopunctatus</i> Theo.....	4
<i>A. (A.) mutilus</i> Edw.....	1
<i>A. (A.) sp.n. near abnormalis</i> Theo.....	9
<i>A. (A.) lamborni</i> Edw.....	3
<i>A. (A.) cumminsi</i> Theo.....	13
<i>A. (A.) natronius</i> Edw.....	11
<i>A. (Banksinella) palpalis</i> Newst.....	11
<i>A. (B.) taeniarostris</i> Theo.....	9

One mouse in the group inoculated with filtrate of the *Aedes* suspension became sick on the fifth day after inoculation and was sacrificed for brain passage. Unfiltered and Seitz-filtered brain suspension was inoculated into separate groups of mice, which, however, remained well, as did the other five mice in the original group receiving the mosquito suspension.

Rhesus 421, which received the suspension of *Aedes* mosquitoes had morning and afternoon temperatures of 104.4°F. on the seventh postinoculation day but did not appear ill. On the morning of the eighth day it was weak and apathetic, refused food, and its coat was roughened, although its temperature was normal. By the afternoon of the eighth day it had become much sicker and its temperature had taken a sharp decline. It was dead the following morning. At autopsy the liver was found to be of normal size and consistency, but pale yellow-brown in color and greasy on section. The stomach contained a large amount of black altered blood. The kidneys appeared swollen and pale. The stained sections of liver showed the characteristic lesions of yellow fever.

Rhesus 421 was bled on the seventh and eighth days, and its serum was subinoculated into mice. Heart's blood and a suspension of liver taken at autopsy were also subinoculated into mice and into rhesus 510. Mice inoculated with seventh-day serum sickened on the sixth day and were all dead by the eleventh day. Mice which received the eighth-day serum also sickened on the sixth day and were all dead by the ninth. Seitz-filtered serum from the heart's blood taken after the death of rhesus 421 caused mice to sicken in seven days. Five of this group were dead by the twelfth day, but one mouse survived. Seitz-filtered liver suspension caused illness in mice beginning on the eighth day. Five of the mice were dead by the fourteenth day. The sixth mouse in this group became paralyzed but lived until the twenty-second day and was then discarded.

Rhesus 510, inoculated subcutaneously May 3, 1944, with Seitz-filtered serum and Seitz-filtered liver suspension from rhesus 421, had a temperature of 105.0°F. on the morning of May 6 and appeared sick. The following day its temperature was subnormal, and it was found dead on the morning of May 8. The gross and microscopic findings were typical of yellow fever. Serum taken May 6 contained virus in a titre of 1 in 3,170,000, and a lot of this serum was preserved by drying while frozen. Third mouse passage virus was used for intracerebral and intraperitoneal protection tests. The intraperitoneal test failed because the 10 per cent virus mixed with normal serum and inoculated intraperitoneally into mice prepared by intracerebral injection of starch did not cause death. However, the intracerebral test was successful: all the mice receiving 1 in 10,000 mouse brain virus with normal serum succumbed, while those receiving the same virus mixed with yellow fever immune serum remained well.

Dried preparations of this strain of virus from *Aedes* mosquitoes and of the one from *A. simpsoni* were used in various experimental studies some months after drying and both have shown the characteristic properties of fully virulent pan-tropic yellow fever virus.

Following the isolation of virus in April 1944, intensive work at Mongiro and in some other forest areas was continued until early July when entomological work in Bwamba was temporarily suspended. Since the beginning of the year 54,125

mosquitoes had been collected in Bwamba, of which 44,667 were taken at Mongiro. Of these, 35,525 had been sent to Entebbe for inoculation into animals. After the isolation of virus in April from *Aedes* spp. reported above, yellow fever was not again encountered.

#### DISCUSSION

The studies made in 1941 (4) in Bwamba County indicated clearly that the vector for yellow fever in man in that region is the semidomestic mosquito *Aedes simpsoni*. The experience of that outbreak offered no explanation of how the *Aedes simpsoni* became infected, but it had already been observed (2, 6) that immunity to yellow fever was more prevalent among persons residing in close proximity to forest than in those residing in open country. This suggested that *A. simpsoni* might acquire its infection from forest animals when they enter plantations in search of food, a possibility which seemed even more probable when virus was again isolated from *A. simpsoni* caught in a forest-edge plantation 11 months after the human population had been effectively immunized (5). Further, a high incidence of immunity to yellow fever was found among all the principal species of monkeys known to inhabit the lowland forests of Bwamba (7). Finally, in 1944, the isolation of virus from a lot of mosquitoes caught in uninhabited forest, and including neither the classic vector *A. aegypti* nor the known vector for man in Bwamba, *A. simpsoni*, established beyond question the existence of an extra-human cycle of virus activity.

Information from the survey of immunity among animals of the Semliki Forest indicates that the extrahuman cycle of yellow fever is endemic, and that monkeys are the principal mammalian hosts (7). However, if the virus is, as it seems, active in the forest more or less continually, it is not necessarily ever-present in any given small locality. Thus the quest of it is most elusive and the forest vector has, thus far, not been discovered. Nevertheless, certain implications as to probabilities may be drawn concerning the 12 species included in the incriminated lot of *Aedes*.

Assuming that the epidemiology of the extrahuman cycle of yellow fever in the Semliki Forest is not a local phenomenon—and at present there is no basis for assuming that it is—several species of mosquitoes in the incriminated lot may be eliminated from general epidemiological considerations on the basis of scarcity or of restricted distribution. These include *A. de-boeri* spp. *de-meillonii*, *A. haworthi*, *A. mutilus*, *A. abnormalis* group *sp.n.*, *A. lamborni*, *A. natronius*, *A. palpalis* and *A. taeniarostris*. Of the species remaining, *A. argenteopunctatus* and *A. cumminsi* have a wide range, but their vector potentialities have not been investigated. *A. apicoargenteus* likewise has wide distribution but was found by Bauer (15) to be incapable of maintaining or transmitting yellow fever virus.<sup>6</sup> *A. africanus* has a wide range and in the experience of Philip (17) and ourselves is an efficient vector of the disease. Therefore, with the possible exception of *A. argenteopunctatus* and *A. cumminsi*—the ability of which to transmit yellow fever remains to be determined—*A. africanus* is the most suspect of the 12 species.

<sup>6</sup> In view of the wide distribution of this species it is felt that specimens from other localities should be tested before it is eliminated from consideration as a possible vector.

Bugher *et al.* (19) found that the principal vectors of jungle yellow fever in Colombia are essentially arboreal and that they attain their highest concentration in the main forest canopy. In Bwamba the information gained from the survey of immunity in monkeys indicates no significant difference in immunity rate between species (such as the baboon, *P. doguera*) which spend much of their time during daylight on the ground, and those (such as *Colobus polykomos*, *Cercocobus albigena*, and *Cercopithecus neglectus*) which rarely descend to the ground and then for short periods only. This situation could be explained by a vector which bites during daylight with equal avidity at ground level and in the forest canopy. No such insect has been found. It could also be explained by a vector which is active in the canopy at any time between sunset and sunrise, during which time both arboreal and terrestrial monkeys are in the trees. *A. africanus* fulfills this requirement in ideal manner. Series of 24-hour catches made simultaneously at ground level and at various heights in the trees have demonstrated clearly that *A. africanus* is the dominant arboreal culicine in the lowland forest of Bwamba (14). Scarce as an adult at ground level, this species is common 50 to 60 feet above ground. It is consistently present in the canopy day after day and females can be taken biting there even under drought conditions. Finally, it has a sharp peak of biting activity in the hour following sunset, as shown by studies here and in West Africa (14, 20). Thus, the indications are that the epidemiology of yellow fever in the forests of South America and Africa may, in general, be similar and that in Africa *Aedes africanus* may be found to be an important forest vector.

In several areas in Uganda it has been observed that the incidence of immunity to yellow fever in monkeys is significantly higher than in the unvaccinated human population in the same region. Moreover, immunity has been found in monkeys in regions where no humans reside, and the isolation of virus from mosquitoes in such a region is here reported. Thus it seems probable that there exists an endemic disease cycle involving forest animals and preferentially arboreal mosquitoes without the participation of the human host. The human host could, and probably does, acquire infection in one or other of two ways: by entering forest and being bitten by the forest vector, as in Colombia (19), or through a semidomestic vector such as *A. simpsoni*. The latter could acquire its infection in forest edge, where it is known to be active, or from animals which leave the forest and enter plantations in search of food. In Bwamba there is present a notable example of such an animal, namely the redtail monkey, *Cercopithecus nictitans mpangae* Matschie. These animals are notorious raiders of plantations. Laboratory tests, as yet unpublished, have shown that they are susceptible to yellow fever, and many immune individuals have been found. It seems probable that they constitute one link between the endemic disease in the forest and the human population.

#### SUMMARY

Yellow fever virus was isolated from *Aedes simpsoni* caught in 1942 in a forest-edge plantation in Western Uganda 11 months after the effective mass immunization of the human population.

The virus was again isolated in 1944 from a lot of 80 *Aedes* mosquitoes, including 12 different species but not including *A. aegypti* or *A. simpsoni*. These mosquitoes were taken in the uninhabited Semliki Forest, indicating an extrahuman cycle of virus activity involving a forest vector.

The implications of the results are discussed with reference to the epidemiology of the disease in forest regions and the means by which the infection reaches man.

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# HISTOPLASMOSIS IN BRAZIL<sup>1</sup>

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The clinical and laboratory diagnosis of Darling's disease (histoplasmosis) has increased in importance owing to the relative frequency with which this disease has been shown to occur in various countries during the past ten years. Following De Monbreun's conclusive experimental demonstration, in 1933, of Rocha Lima's old hypothesis that histoplasmosis was really a mycosis, an awakened interest in the diagnosis of this disease has been clearly indicated by the increase in the number of cases described. Two excellent reviews of the historical aspects and the contemporary knowledge of histoplasmosis are found in the articles of Negroni (1940) and Meleney (1940). The subject was amplified in a recent paper, by Moore and Jorstad (1943). A short summary of the more important work on this visceral mycosis is given below, followed by a brief account of the cases found in Brazil.

## HISTORICAL

While investigating the presence of kala-azar among the natives of the Panama Canal Zone, Darling (1906) discovered a disease entity whose symptoms and pathology were similar to those of kala-azar, but in which the visceral lesions contained a parasite different from *Leishmania*. Darling (1908) later concluded that the organism was a new protozoon, and named it *Histoplasma capsulatum*.

Rocha Lima (1912) examined Darling's preparations and suggested that *H. capsulatum* might be a fungus of the same group as *Cryptococcus farciminosus*, the causative agent of epizootic lymphangitis. This hypothesis was accepted by Darling.

The disease was not described again until 1926, at which time Watson and Riley (1926) reported the infection occurring in a woman in the state of Minnesota, U. S. A.

De Monbreun (1934) confirmed the hypothesis that the disease was a mycosis. He isolated a fungus from the blood and spleen of a child with histoplasmosis, whose case had been described by Dodd and Tompkins (1934). The organism had cultural and morphological characteristics which indicated that it belonged to a new genus. He continued, however, to use the name assigned by Darling. He was able to infect a rhesus monkey with the fungus and to reproduce in this animal a clinical picture similar to that observed in human cases.

Hansmann and Schenken (1934) simultaneously described a fatal systemic mycotic infection with extensive cutaneous lesions. They isolated a fungus which they considered as belonging to the *Sepedonium* genus. This specimen was

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later studied by Moore (1934) and was first regarded by him as a new genus, *Posadasia*, but was finally identified by Redaelli and Ciferri (1934) as *H. capsulatum*. Ciferri, Redaelli, and Visocchi (1938) completed the mycological study of this species, creating for it a new family, *Histoplasmaeaceae*, in which they included *Cryptococcus farciminosus*, Rivolta and Micellone, 1883, and *Cryptococcus muris*, Short, 1932.

Redaelli and Ciferri (1934a) were also able to produce experimental *Histoplasma* infection in white rats, guinea pigs, and rabbits. As the result of these experimental studies it was established that the disease process caused by *Histoplasma* infection is essentially a systemic reticulo-endotheliosis.

De Monbreun (1939) first observed the spontaneous occurrence of this disease in a domestic dog, and obtained from the sick animal a new strain of fungus, which he proved to be identical with one which he had isolated from a human case. He was also able to reproduce the disease experimentally in puppies. He suggested that the dog might serve as a natural host for the organism, and that the infection might be transmitted to man either by blood-sucking vectors or by direct contact.

Thuringer (1944) and Callahan (1944) recorded, respectively, the second and third cases of natural canine infections, but they made no attempt to cultivate the parasite. None of the three animal cases reported was associated with a human infection.

Tager and Liebow (1942) and also Parsons (1942) showed that the young white mouse is very susceptible to *Histoplasma* infection, especially when inoculation is made by the intravenous route. Simson and Barnetson (1942), isolated a human strain of *Histoplasma* in South Africa, which, although morphologically identical with *Histoplasma capsulatum*, was not pathogenic to animals and did not grow at 37°C.

Moore (1941) was successful in cultivating *H. capsulatum* on the chorioallantoic membrane of the developing chick embryo. He made a detailed study of the conversion of the mycelial, or saprophytic, form of *Histoplasma* into the yeastlike, or parasitic form. He confirmed previous *in vitro* observation of the segmentation of the aerial hyphae, followed by the enlargement of the separate elements and their final fission, a process which is similar to the formation of arthrospores, or oidia. He also observed the endosporulation of the tuberculate cells which finally became converted into clusters of yeastlike spores. He concluded that these hyphospores should be considered "ascus-like" structures, probably of a degenerated nature.

Duncan isolated the fungus in England from a case notified by Derry *et al.* (1942). The victim was a soldier who had probably contracted the disease in the Sudan or in India. For cultivation purposes Duncan used Allison and Ayling's (1929) potassium tellurite and copper sulphate bacteriostatic medium to inhibit the prejudicial growth of a staphylococcus contaminant.

Crumrine and Kessel (1931) had reported a human case without splenomegaly, but with intensive gastrointestinal and glandular involvement. Broders *et al.* (1943) described a fatal case of histoplasmosis in which the initial lesions probably



occurred in the throat. The fully developed clinical picture was consistent with that of a septic endocarditis, and autopsy revealed a vegetative endocarditis of the mitral valve and histoplasmas in the heart lesions and in the adrenals. The diagnosis had initially been made during life by means of a liver biopsy. Of great interest is the fact that liver specimens taken at autopsy showed neither parasites nor lesions.

This would seem to indicate that *Histoplasma* infection does not necessarily present the invasive and generalized features observed in classical cases, but that, on the other hand, it may at times be anatomically focal in character.

Zarafonitis and Lindberg (1941), and Van Pernis, Benson, and Holinger (1941), independently devised similar methods for the diagnosis of histoplasmosis by intradermal tests. The antigens comprised either dead suspensions of the yeast-like form of the fungus, or soluble substances from the filtrate of old mycelial-form cultures. The specific value of these tests has not been definitely proven.

Alonso and Freijó (1944) reported the first recognized case of histoplasmosis in Uruguay, the diagnosis being based on examination of a biopsy specimen. The patient made a complete recovery following the use of sulfadiazine therapy. In several other cases described in the literature, however, sulfonamides and chemotherapeutic agents such as arsenicals, antimony, iodine, and bismuth, have failed to effect a cure. It may be stated that no specific treatment for the disease has as yet been generally recognized.

The cases of histoplasmosis so far reported have occurred in widely separated regions in this order: Central America, Philippine Islands (?), United States, Austria, Java, Argentina, Brazil, South Africa, England, Mexico, Venezuela, Portugal, and Uruguay. The greatest number of confirmed cases has been found in the United States, where there are at least two zones in which the disease exists in endemic form. These endemic zones lie in Missouri (Beamer et al. 1944) and Michigan (Parsons and Zarafonitis, 1945).

#### OCCURRENCE IN BRAZIL

The first undoubted Brazilian case of histoplasmosis was diagnosed by Villela and Pará (1941) in 1939, but the case was not reported until 1941. The diagnosis was based on the histopathological examination of a viscerotomy liver specimen in which typical lesions and parasites were seen. Between the years 1939 and 1944, a total of 169,808 liver specimens were collected and examined during the routine histopathological control of yellow fever, and among these specimens four additional cases of this visceral mycosis were encountered (fig. 1). The clinical histories of these cases and a description of the liver lesions are given below. The clinical and epidemiological data for each case were obtained only after the histopathological diagnosis had been made, consequently complete and more precise information is lacking.

Three other cases have been found by biopsy or autopsy in Brazil: two, previously reported, by Almeida and Lacaz (1939) (1941), and one by Duarte in 1944 (personal communication). The former are questionable cases and the later report has not yet been published. This case however was a fatal one and was

discovered at autopsy. Characteristic lesions and parasites were found only in the lymphoid structure of the small intestine, in the brain, and in the meninges, where an intense, acute inflammatory process was observed. There was no evidence of liver or spleen involvement.

DIAGNOSIS OF HISTOPLASMOSIS CASES BASED ON EXAMINATION OF  
VISCEROTOMY LIVER SPECIMENS

*Case No. 1—H. M. S.*—A three-year-old mulatto boy who died on July 7, 1939, in Recreio, Minas Gerais (previously reported by Villela and Pará, 1941).

*Clinical data.* The child had been born and brought up in a habitation in which several families lived together. There was intimate contact with both dogs and cats. He was presumably in good health until two months before death. The onset of his illness was characterized by fever, weakness, and anorexia. He became progressively emaciated. One month later an attack of "bloody diarrhea" occurred, which lasted several days and for which the patient was given a syrup containing iodine and tannic acid, an oral antidyentery vaccine, and a tonic containing vitamins A and D. One week before death his skin became jaundiced, and "spots" appeared on several different parts of the body. He was not confined to bed until three days before death. No animal infection nor other human case could be revealed by subsequent investigations.

*Liver pathology*—Lab. No. 172092. There is a discrete alteration in trabecular structure, but the hepatic cells are normal in appearance, and there is no evidence of necrosis. Fatty degeneration is marked, with the formation of large and medium-sized vacuoles. Rare multinucleated cells are seen in the peripheral zone of the lobules. In addition to the marked hyperplasia of the Kupffer cells there is an intense infiltration of the portal spaces with fibroblasts, lymphocytes, endothelial and polymorphonuclear leukocytes. There is no granuloma formation or fibrosis. Numerous parasites can be seen in almost all of the Kupffer cells and in the cells infiltrating the portal spaces. The parasitized cells are diffusely scattered throughout the hepatic lobule, but are most numerous in the portal spaces.

*Case No. 2—B. H. R.*—A 19-months-old colored, male child, who died Sept. 7, 1943, in Xiririca, São Paulo.

*Clinical data.* The patient was born in a straw hut situated about four kilometers from Xiririca. The mother died during labor, and the infant was raised on cow's milk and porridge. His father is alive and healthy. Little is known about the onset of the child's fatal illness, since the history was complicated by the presence of a persistent rectal prolapse which appeared at the age of two months, and, in addition, a helminth infestation. He failed to gain weight normally, and suffered from anorexia, alternating constipation and colicky diarrhea, and insomnia. Concerning the events immediately preceding his death, it is known only that dark macules appeared on the soles of the feet, the thighs, and the trunk two days before demise. During life, the child had often been taken to a neighboring house, some two kilometers distant, where there were three dogs. Subsequent investigation of these animals resulted in the isolation of a strain of *Histoplasma*.

This data is described in a subsequent section. No other human cases were detected through the field inquiry.

*Liver pathology*—Lab. No. 300484. Necrosis and fibrosis are absent and there is no alteration in nuclear or trabecular structure. There is a fatty infiltration, consisting of large vacuoles, and a slight hyperplasia of the Kupffer cells. In the portal zone there is a definite, variably outlined, granulomatous reaction which consists of an intense proliferation of epithelioid cells and fibroblasts and an in-



FIG. 1. MAP OF BRAZIL SHOWING LOCALITIES WHERE THE FIVE HUMAN CASES OF HISTOPLASMOSIS OCCURRED

filtration of monocytes, lymphocytes, and plasma cells. Giant cells of the Langhans type are also in evidence. Within the granulomata numerous parasites are visible, sometimes free, but generally intracellular. Some of the *Histoplasma* agglomerations are surrounded by a halo, which doubtless represents an achromatic capsule common to these organisms. Such structures have the appearance of *Toxoplasma* "pseudocysts," and are found most frequently in parasitized giant cells.

*Case No. 3—B. A. A.*—A six-year-old colored girl who died on June 11, 1944, in Itapé (Ilhéus Region), Bahia.

*Clinical data.* The child was born at term, in a small mud hut in the village of Itapé. At the age of one month she had measles, followed almost immediately by alastrim. At five years of age, early in 1943, she experienced intermittent attacks of nocturnal fever over a period of two months and, simultaneously, her parents noted the appearance of two swollen glands in the left inguinal region which gradually increased in size. Other swellings appeared on the right side, and subsequently in the axillary, cervical, and postauricular regions. Six months later one of the original buboes in the left inguinal region, having reached the size of a small lemon, ruptured and released a purulent cream-like secretion. The open bubo ulcerated and the crater, covered with a fetid yellow secretion, extended almost to the vulva. One month later she was taken for the first time, to a doctor who prescribed medicines to combat her anemia, sulfanilamide, and the local application of an ointment containing iodine and methyl salicylate. She failed to improve, however, and buboes continued to develop. Anorexia appeared, and, during the last two weeks of her life, she was confined to bed because of marked lassitude and weakness. No animal case or other human case was discovered by the field investigation.

*Liver pathology*—Lab. No. 320726. The trabecular structure is slightly altered. A large number of binucleated hepatic cells are present. There are some foci of lytic necrosis, for the most part situated in the periphery of the lobules, and varying considerably in size. An intense fibroblastic proliferation and lymphocytic infiltration can be seen in the foci. There is a generalized endothelial hyperplasia, discrete passive congestion, and, as well, a diffuse lymphocytic infiltration. In the portal spaces there occurs early connective tissue hyperplasia and mononuclear cell infiltration. In some lobules the portal fibrosis shows an invasive tendency. There is definite bile stasis, leading to the formation of small bile cylinders within the hepatic cells, particularly in the portolobular zones. Numerous clusters of Histoplasma are visible in the necrotic foci, in the portal spaces, and rarely in histiocytes not involved with the lesions.

*Case No. 4—C. B. O.*—A three-year-old girl who died on April 28, 1939, in Itapé (Ilhéus Region), Bahia.

*Clinical data.* This case was overlooked in the original routine examination of the histopathological material, but was discovered on re-examination of the 442 liver specimens which had been received from the locality of Itapé up to July 1944, when case No. 3 was diagnosed. We were unable to obtain any clinical data on this case except that the victim had died during a measles epidemic. Five of her brothers died at the same time, and liver specimens were obtained from two of these. Both were negative for histoplasmosis.

*Liver pathology*—Lab. No. 166624. The nuclear and trabecular structures are normal. There is a discrete fatty infiltration in the form of large droplets. Various-sized foci of lytic necrosis are present, which, when combined with the fibroblastic proliferation and infiltration by mononuclear and polymorphonuclear cells, give the focal lesions the appearance of microabscesses. In the portal spaces a marked proliferation of fibroblasts, plasma cells, and phagocytes can be

seen. A mild biliary hyperplasia is present in the peripheral zone. Passive congestion is slight. In the necrotic foci and in the portal spaces, various heavily-parasitized cells are seen, but they are much less pronounced than in the other cases of our series. In this specimen there are but few parasitized cells outside the focal lesions. There is a discrete proliferation of connective tissue in the portal spaces.

*Case No. 5—S. R. E. S.*—A nine-year-old female mulatto who died on July 24, 1944, in João Alfredo, Pernambuco.

*Clinical data.* The patient's family moved to João Alfredo only a few months before her death, having previously lived in the neighboring município of Limoeiro since her birth. The child had been weak and anemic since infancy, and for a long time had a chronic cough, which had been productive of abundant yellow sputum, and had caused her voice to become hoarse and reedy. Her anemia and emaciation became progressive, and by July 1944, her health had become seriously impaired. Shortly thereafter she became even worse, developing nausea, vomiting, and mild afternoon fever. Inguinal, cervical, and axillary adenitis developed, and it is possible that she also had ulcerations of the nasopharyngeal and epiglottis mucosa. During her last two weeks she was in a state of frank cachexia, and, as a terminal event, she developed icterus. No information was obtained about her parents' health or the possible existence of other associated cases.

*Liver pathology*—Lab. No. 324925. There is no change in trabecular structure. Fatty degeneration is present in the form of medium-sized droplets. In some of the lobules there is necrobiosis of cells in the central zone, the nuclei being pyknotic. Small foci of lytic necrosis are present, showing lymphocytic infiltration with fibroblast and Kupffer cell proliferation. There is also lytic necrosis in the portal zone, with histiocytic hyperplasia and marked polymorphonuclear leukocyte infiltration, thus having, not uncommonly, the same appearance as the microabscesses seen in pyelophlebitis. Diffuse and discrete biliary stasis is present. In some cases a mild connective tissue hyperplasia can be seen in the portal spaces. Numerous clusters of histoplasma are to be found in the necrotic foci, the microabscesses, the portal spaces, and within the reticulo-endothelial cells and polymorphonuclear leukocytes. On the contrary they are only sporadically found in cells other than those comprising the focal lesions.

#### SUMMARY OF CLINICAL DATA

\* The conditions under which the epidemiological investigations were carried out made it impossible to obtain more than the barest details of clinical history. Notwithstanding, certain comments are justified.

There were three cases with chronic symptoms which lasted for almost a year or more. Only Case 1 can be considered as the acute type of histoplasmosis, its duration being less than sixty days.

All of the five victims were children, two boys and three girls, from nineteen months to nine years of age. Four were colored or mulatto. None received adequate medical attention, the diagnosis of histoplasmosis having been established only by histopathological examination of post-mortem liver specimens.

On analyzing the clinical data, one is struck by the persistence, in all phases of the disease, of three symptoms: anemia, anorexia, and progressive emaciation leading to cachexia and death. Two different types of fever were manifest in all of the cases: either remittent in the early stages of the disease, or subcontinuous low-grade fever with afternoon rises once the disease process had become fully developed. Another frequent symptom was diarrhea, either simple or bloody, and occurring at intervals with or without fever. The typical glandular form of the disease was seen in the third case of our series. In another case, lesions of the epiglottis and nasopharyngeal mucosa, associated with a chronic productive cough, would indicate that the respiratory tract had been seriously invaded.

We were unable to learn whether hepatomegaly and splenomegaly occurred in any of our cases; however, functional involvement of the liver was probably present in those which developed cutaneous icterus during the terminal stage. A symptom noted in two of the cases is the appearance, a few days before death, of purple spots in the skin which might be regarded, since they occurred in children, as a manifestation of toxic purpura.

#### SUMMARY OF PATHOLOGICAL DATA

In general, the pathology of the liver specimens from our five cases of histoplasmosis corresponds to that which has been previously described by other authors.

In the first case, the hepatic lesions indicate a typical reticulo-endothelial inflammatory process, similar to that produced by visceral leishmaniasis.

In the second case, the histiocytic hyperplasia tends to be localized, giving rise to true granulomata whose morphological characteristics can be confused with those of coccidioidal granuloma (fig. 2). In the three remaining cases, the most striking lesion is focal necrosis, occurring concomitantly with reticulo-endothelial hyperplasia of moderate intensity. The necrosis (fig. 3) is of the lytic type similar to that usually produced by infarcts. Two of the cases showed inflammatory and necrotic foci having the appearance of microabscesses (fig. 4).

Discrete portal fibrosis was a common finding. We noted, in four of the five cases, that the infiltrative and proliferative changes were mainly localized around the portal spaces. One of the cases had very few lesions elsewhere. This predominance of portal-space or periportal lesions has been interpreted, we believe correctly, to indicate that the live involvement occurred via the portal vein and was, therefore, of intestinal origin. It is our opinion, however, that the localization of lesions in the peripheral zone of the liver lobules indicates only that the liver was infected secondary to intestinal lesions, and does not warrant the conclusion that the oral route was necessarily the original way of entry. Intestinal lesions might come about as the result of a systemic blood-borne infection, or more probably, through infection of the respiratory tract with subsequent coughing up and swallowing of infectious material.

The parasites seen in the liver sections from all five patients have the morphology characteristic of *Histoplasma* in tissues; i.e. they appear as rounded, en-

capsulated yeast-like bodies, varying in size from 1 to 5 micra. Within the cell is an eccentric chromatic splotch, or even a well-defined nucleus, adherent to the

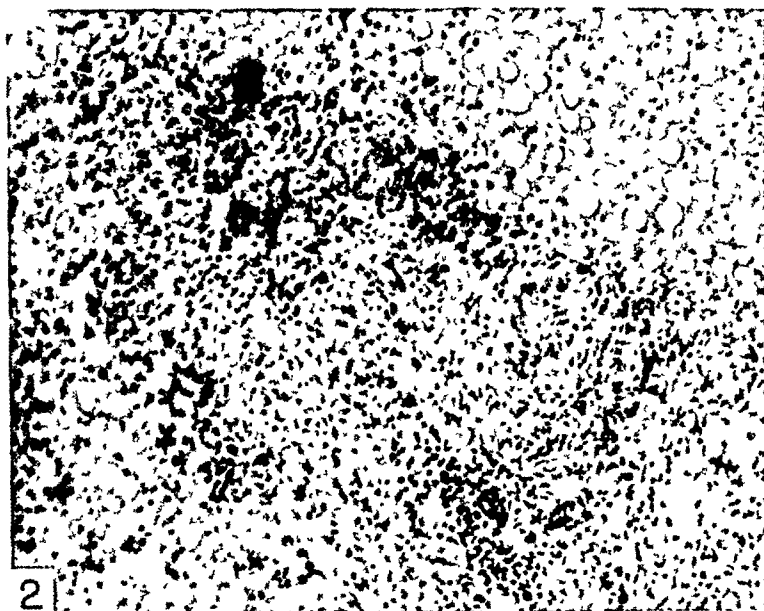


FIG. 2. NEAR-PORTAL GRANULOMA, OF THE INFECTIVE TYPE, CAUSED BY HISTOPLASMA CAPSULATUM

Human liver, 2nd case. Hematoxylin-eosin.  $\times 130$ .

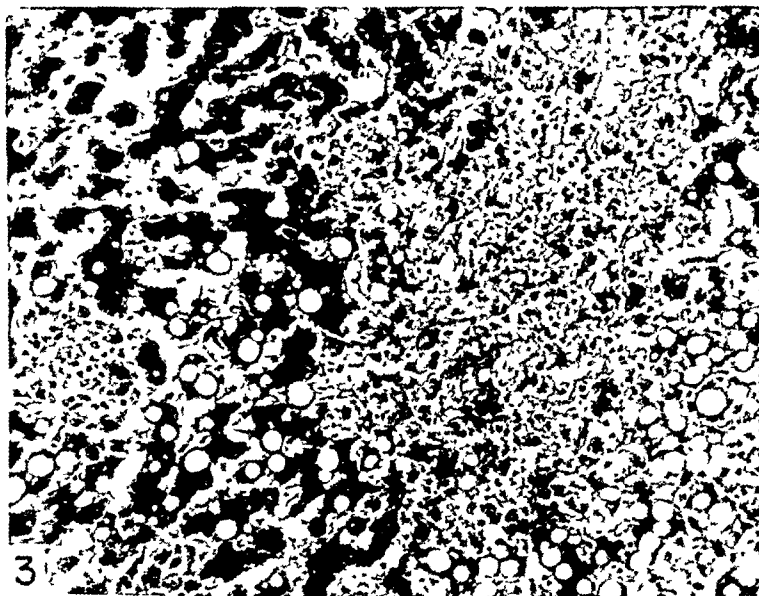


FIG. 3. NECROTIC FOCI SHOWING CELLULAR INFILTRATION AND PROLIFERATION WITH PARASITES

Human liver, 4th case Hematoxylin-eosin.  $\times 130$

cell membrane. Aside from the presence of a vacuole and capsule which is not stained by ordinary methods, the organism shows no other special structural

features and, in general, can easily be distinguished from *Leishmania* or *Toxoplasma*. In the cases described, they were found for the most part in histiocytic cells, but they also occurred in connective tissue cells and even in the epithelial

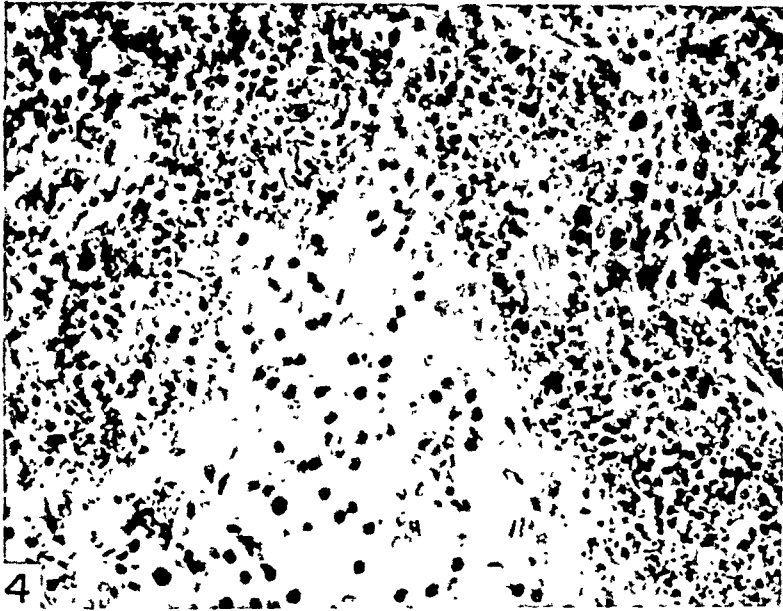


FIG. 4. EXTENSIVE INFLAMMATORY REACTION IN A PORTAL SPACE FORMING A PILEPHLEBITIC MICROABSCESS

Various histoplasmas can be seen inside histiocytic cells. Human liver, 5th case. Gram-Weigert.  $\times 300$

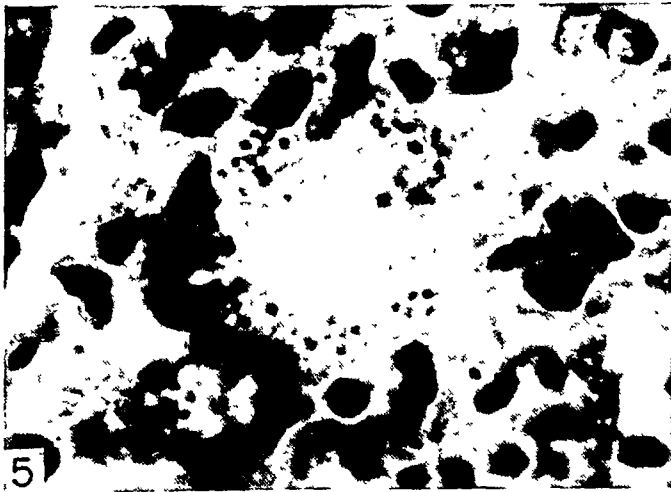


FIG. 5. LANGHANS TYPE GIANT CELL, WITHIN A GRANULOMA, CONTAINING SEVERAL CLUSTERS OF CAPSULATE YEASTLIKE SPORES OF HISTOPLASMA  
Human liver, 2nd case. Heidenhain.  $\times 1200$

cells of the liver. They were also seen within the epithelioid and giant cells of the granuloma (figs. 5 and 8).

The parasitized cells, which are generally loaded with histoplasmas, were



hypertrophic and sometimes necrobiotic. The organisms were almost always intracellular, but on occasions many of them were observed free in the sinusoids (fig. 6). It is our impression that the presence of extracellular organisms is due to the rupture of heavily parasitized cells.

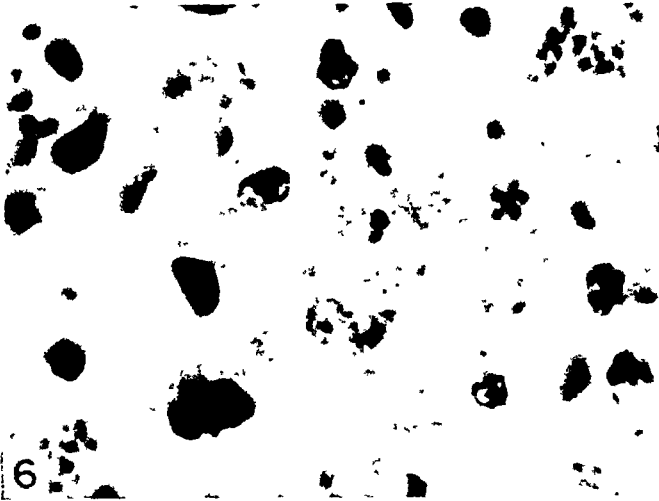


FIG. 6. SOME AGGLOMERATIONS OF HISTOPLASMAS, APPARENTLY FREE, WITHIN THE SINUSOIDS  
Human liver, 4th case. Giemsa.  $\times 1200$

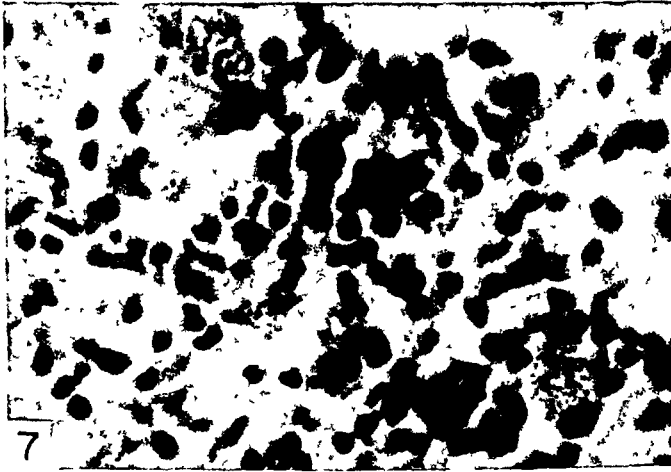


FIG. 7. "PSEUDOCYST" RESEMBLING CLUSTERS OF HISTOPLASMA LOCATED INSIDE A GRANULOMA

This form can be better distinguished among the clusters situated within the giant cell.  
Human liver, 2nd case. Hematoxylin-eosin.  $\times 650$ .

A rather rare and curious finding was the formation of a halo surrounding the agglomerations of histoplasmas, particularly in the giant cells. Morphologically the formation closely resembled the "pseudocysts" of the *Toxoplasma* and could be distinguished from them only by differences in internal structure of the elements. It is possible that such formations result from the more or less complete fusion of individual capsules (fig. 7).

In tissue sections the histoplasmas, with the exception of the capsule, show variable affinity for the basic dyes employed in current staining procedures: i.e., hematoxylin-eosin, Masson's trichromic, Van Gieson, Goodpasture, Gram-Weigert, and Giemsa.

Of practical interest are our observations made with the Krajian (1943) Gram-modification and the Foshay (1931) method. Stained by either of these techniques, *Histoplasma* can easily be distinguished from *Leishmania*. With the former, the histoplasmas appear blue-violet or blue-green, while the leishmanias are pale red. With the Foshay method, *Histoplasma* stain blue and *Leishmania* appear mauve.

The Heidenhain method and most of the silver impregnation techniques bring out the *Histoplasma* capsule very clearly (fig. 9).

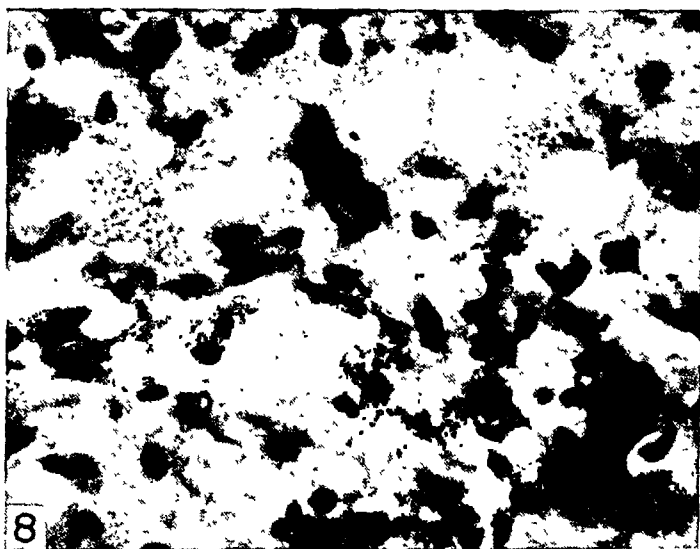


FIG. 8. VARIOUS GROUPS OF HISTOPLASMAS ARE SHOWN PARASITING CONNECTIVE TISSUE CELLS WITHIN A FIBROTIC ZONE  
Human liver, 3rd case. Krajian.  $\times 650$

#### THE ISOLATION OF "HISTOPLASMA CAPSULATUM" FROM A DOMESTIC DOG

Unsuccessful attempts were made to isolate *Histoplasma* from the exhumed remains of the second and third human patients, and also from a dog which had been in close contact with the first patient. Subsequent to the field investigation, however, we received three live dogs from the home of patient No. 2. These dogs were anemic and emaciated mongrels. Culture and microscopic examination of blood specimens taken from them immediately after arrival were negative. Several of their fleas were examined, also with negative results. Less than a month later, all three animals died of intercurrent *Salmonella enteritidis* infection. The autopsies and histopathological examination of material from two of them showed no evidence of histoplasmosis.

The necropsy on the third dog, however, revealed a liver with yellow blotches and slight congestion, massive congestion of the small intestine, and splenomeg-

aly. The mesenteric lymph nodes were slightly enlarged, and there were small areas of consolidation in bases of both lungs. Specimens of the brain, sternal bone marrow, right and left lungs, liver, gall bladder, spleen, small and large intestine, omentum, and the mesenteric lymph nodes were preserved for future study.

Histopathological examination revealed: the presence of a variable number of microfilaria in all of the tissues examined, with the exception of the brain; liver abscesses containing filaria; acute enteritis; reticulo-endothelial hyperplasia and fibrosis of the spleen and lymph nodes; leukocytic infiltration, hyperemia, and cellular necrosis in the lungs. A few organisms with morphology similar to that of *Histoplasma* were found, occurring in small groups. They were evident only

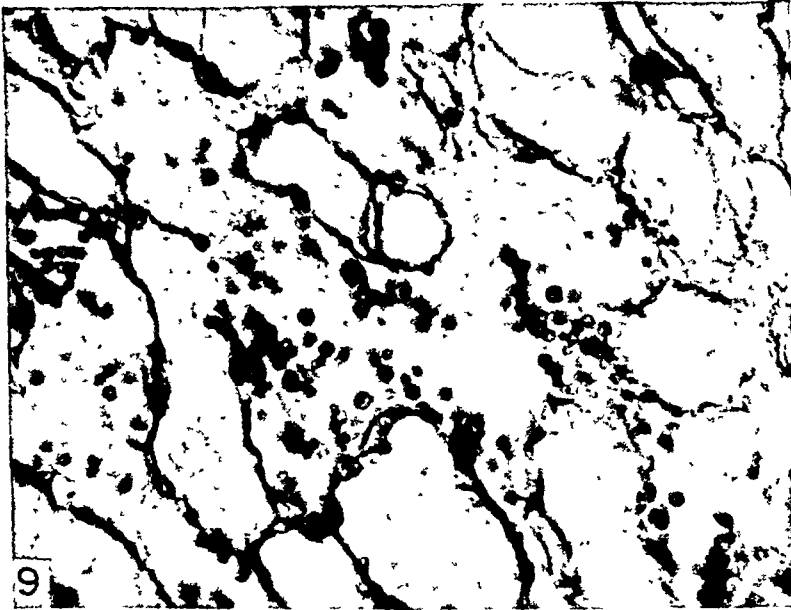


FIG. 9. HISTOPLASMA BODIES WITH CAPSULES CLEARLY VISIBLE AFTER SILVER IMPREGNATION

A slight thickening of the reticular fibers can be observed. Human liver, 1st case. Rio Hortega reticular method.  $\times 850$ .

in sections of the lungs, being found within the phagocytic cells of the interstitial tissue or those in the lumen of the bronchioles (figs. 10 and 11).

Autopsy material, which had been kept in the refrigerator for a week, was inoculated into appropriate culture media and also into white mice. The mice were injected by the intraperitoneal route with material which had first been treated with a 0.05 per cent copper sulphate saline solution for twenty-four hours at room temperature, triturated in a mortar, and suspended in dextrose broth. Suspensions were made of tissue from the mesenteric lymph nodes, the spleen, the liver, and the lungs. Each mouse received 0.5 ml. of inoculum intraperitoneally. Six 21-day-old mice were used for each suspension. The mice were observed for about 200 days following inoculation, and were then sacrificed. Nothing abnormal was revealed by the pathological and microscopical examination of their viscera.

Fragments of fresh material from the lung, liver, spleen, and lymph nodes were planted on culture media and maintained under aerobic conditions at room temperature and also at 37°C. The following media were used: Sabouraud's agar, Blood-Sabouraud's agar, Sabouraud's copper sulphate (0.05 per cent) agar, and laked blood-dextrose broth. All of the tubes and flasks were observed for a

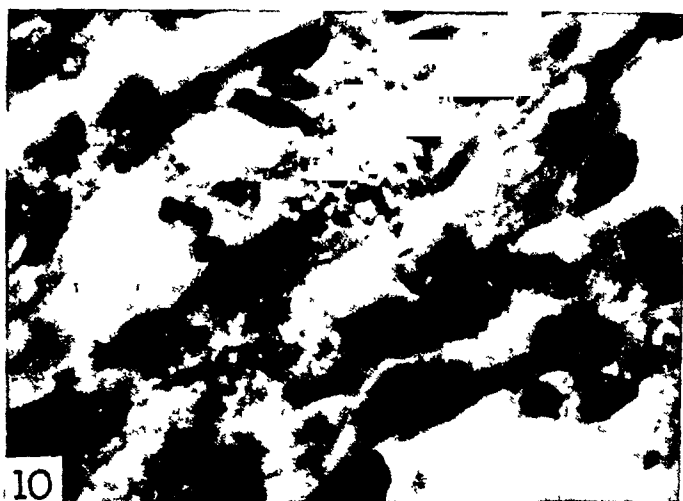


FIG. 10. A CLUSTER OF HISTOPLASMAS INSIDE A MACROPHAGE CELL SITUATED WITHIN THE LUNG INTERSTITIAL TISSUE  
Dog with natural infection. Heidenhain  $\times 1100$

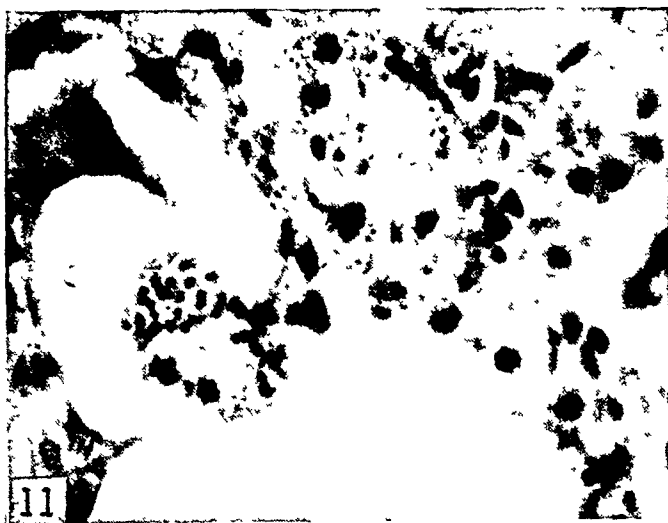


FIG. 11. A GROUP OF HISTOPLASMA BODIES ON CELLULAR DEBRIS IN THE LUMEN OF A BRONCHIOLE, ALSO INFILTRATIVE REACTION IN NEIGHBORING LUNG TISSUE  
Dog with natural infection. Giemsa  $\times 800$

period of one month, and showed, with the exception of the copper sulphate medium, an abundant growth of *Salmonella enteritidis*. The identification of this organism was performed by biochemical and serological tests. In only one tube did a fungus culture appear, this after eight days at room temperature. The tube contained Sabouraud-copper sulphate medium on which lung material had

been planted. Microscopic examination of the organism revealed morphological characteristics consistent with those of *H. capsulatum*. A transplant of this growth to Sabouraud's agar was made for mycological study. The organism was designated as "Strain PCTA Dog Lung".

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FIG. 12. "PCTA" STRAIN OF HISTOPLASMA CAPSULATUM ISOLATED FROM THE LUNG OF A DOG ASSOCIATED WITH THE SECOND CASE DESCRIBED  
Culture in Sabouraud's agar, 10 days old, at room temperature. Natural size.

#### MYCOLOGICAL OBSERVATIONS

In Sabouraud's medium or in serum-dextrose agar this fungus began to grow after thirty-six hours' cultivation at room temperature. After eight days it had become well developed, and appeared as a mycelial culture, flat, matte-white in color, and similar to rat's fur in appearance, tending to spread over the surface of the medium to which it had become firmly adherent (fig. 12). The mycelial culture could not be emulsified in saline. On microscopical examination (figs. 13 and 14) it appeared as a well-developed hyalin mycelium with separate hyphae, generally showing lateral branches of almost uniform thickness (1 to 3 micra, with a mean value of 2.20 micra). In some cases the filaments were segmented and

contained ovoid cells, giving rise to the so-called "racquet" mycelia. The hyphae occasionally showed terminal buds limited by a septum slightly thicker than usual. These were considered to be chlamydospores in the process of formation. Rarely, these structures were intercalary. Small, spherical or oval spores, 1 to 2

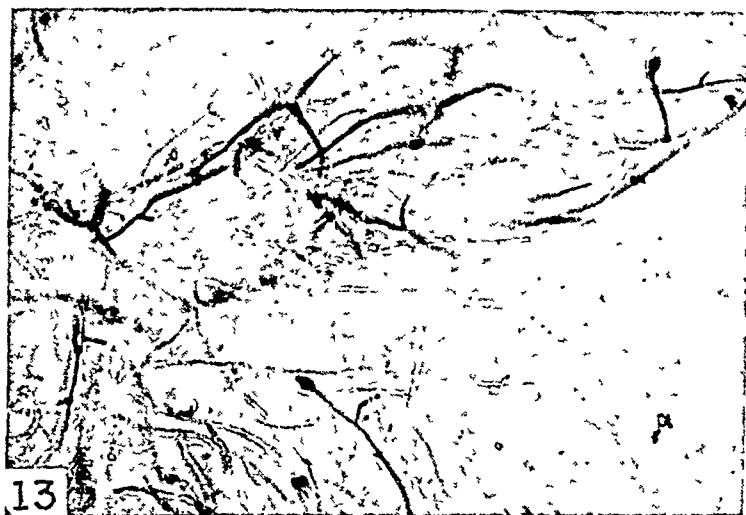


FIG. 13. CHLAMYDOSPORES IN FORMATION AND CONIDIA. MYCELIAL FILAMENTS, SEPTATE AND WITH LATERAL BRANCHING

From the same culture as fig 12. Gueguen stain.  $\times 260$



FIG. 14. APPEARANCE OF THE SMOOTH TYPE CHLAMYDOSPORES OR "ASCUS-LIKE" CELLS AND MYCELIAL GROWTH FROM THE ISOLATED STRAIN

Sabouraud culture 10 days old at room temperature. Gueguen stain.  $\times 260$

micra in size, having a simple membrane, were frequently seen around the hyphae. These were identified as conidia (fig. 13) and were either attached directly to the hyphae or were pedicellate.

Usually free, but at times connected laterally or at the end of an aerial hypha, were large spores, having a double membrane and, almost always, containing a

granular endospore. Such spores ordinarily measure 5 to 20 micra, with a mean value of 14.40 micra, and are the fully developed chlamydospores or hypnospores (figs. 14 to 17). They increase in number with the age of the culture, and, when tuberculated, they are the characteristic morphological feature of *H. capsulatum*. In our particular strain, the majority of the hypnospores were smooth, and the tuberculated cells, when they did occur, were always small (5 to 10 micra). The inner portions of some of the hypnospores were loculated. In 3- to 5-day-old

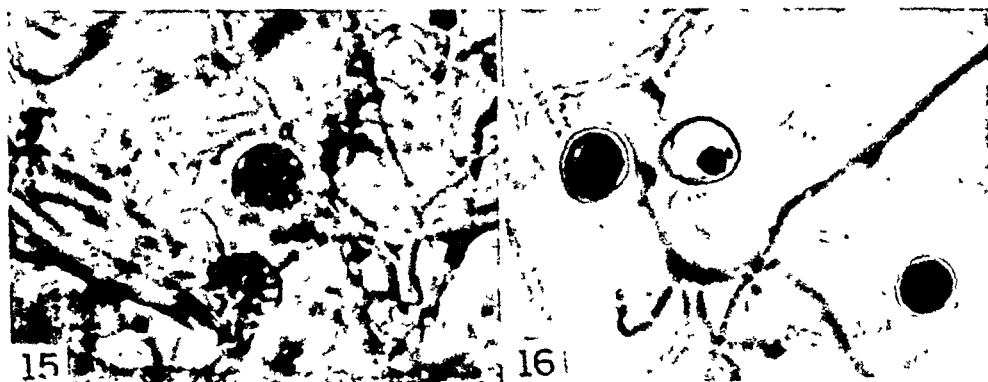


FIG. 15. CHLAMYDOSPORES WITH SMOOTH TYPE EPISPORE, AND GRANULOUS ENDOSPORE FROM THE ISOLATED STRAIN

Culture 10 days old at room temperature. Gueguen stain.  $\times 700$

FIG. 16. SMOOTH CHLAMYDOSPORES, ONE OF WHICH, WITH ITS INNER PART ALMOST EMPTY, IS PROBABLY UNDERGOING DEGENERATION

The lower cell is seen in intercalary position on an aerial hypha. Sabouraud culture 15 days old at room temperature. Gueguen stain.  $\times 700$ .



FIG. 17. SMALL TUBERCULATE CHLAMYDOSPORE FROM THE SAME STRAIN  
Sabouraud culture 15 days old at room temperature. Gueguen stain.  $\times 1000$

cultures it was not uncommon to observe the extrusion of mycelial filaments from within the body of the hypnospore. Evidence of endosporulation was seen on rare occasions in old, well-developed cultures.

Glycogen and fat could be demonstrated in the vegetative tubes by the Gueguen method of staining. By this technique the hypnospores were also shown to be rich in fat, and their episporos became heavily stained with blue.

In liquid media the fungus grew initially in single radiating colonies of a yellow-

ish-white colour. The mycelia had relatively few frutification elements. After about two weeks of growth, the single colonies agglomerated into a gelatinous mass which usually spread out onto the surface of the medium, forming a matte-white pellicle with aerial hyphae. Sometimes it formed a sediment, leaving a clear supernatant fluid.

All attempts to cultivate the PCTA strain at 37°C. proved unfruitful. The fungus grew well, however, at temperature of 25°–30°C. on the following culture media having a pH of 7.2 to 7.4: Blood-Sabouraud's agar, Csapek media, dextrose broth, and liver infusion broth.

Numerous unsuccessful attempts have been made to demonstrate pathogenicity of this strain for white mice. The fungus has also been proven non-pathogenic when inoculated intravenously into a cebus monkey and into guinea pigs by both intratesticular and intravenous routes.

A more detailed comparative study of the strain is in progress, and the results will be the subject of a future publication.

#### SUMMARY

Five cases of "histoplasmosis of Darling" occurring in Brazil are described. The diagnosis was based on histopathological examination of human viscerotomy liver specimens collected between 1939 and 1944 inclusive. The data on the first case have been published previously.

Among a total of 169,808 liver specimens examined, only these five cases of histoplasmosis were found. This would seem to indicate that the disease is not frequent in Brazil; but allowance should be made for the inherent limitations of viscerotomy.

The essential histopathological liver lesions in these cases include marked reticulo-endothelial hyperplasia, granuloma formation, focal necrosis, and micro-abscesses. These have been described by other authors as occurring in this disease.

*H. capsulatum* was isolated from the lung of a house dog which had been closely associated with one of the human patients. This is the fourth reported case of natural histoplasmosis in dogs and, to our knowledge, it is the first canine case to be linked directly with a human infection.

A brief description is given of the morphological and cultural characteristics of the newly isolated strain.

#### ACKNOWLEDGMENTS

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# ORAL EMETINE IN THE TREATMENT OF INTESTINAL AMEBIASIS

## A PRELIMINARY REPORT

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Emetine, one of the several alkaloids contained in the root of *Cephaelis ipecacuanha*, was first used in the treatment of dysentery by Helvetius in 1685 in the form of the powdered dried root. Over a hundred years later Pelletier (1) succeeded in separating emetine from the other alkaloids and as such it was successfully employed as a therapeutic agent by Bardsley in 1829 (2). In 1891 Walsh (3) treated cases of dysentery with the mercuric iodide salt of the drug and claimed good results. At first, emetine was used in all types of dysentery and as a consequence there were many failures. When amebic dysentery was established as an entity, the effectiveness of the alkaloid as a therapeutic agent for amebic dysentery was firmly established.

Vedder (4) in 1911, published a report covering preliminary experiments undertaken to test the efficacy of the alkaloid, and stated that in dilutions of 1 to 100,000 it was lethal to the amebae *in vitro*. Further studies carried out by Vedder in 1912 (5) and also in 1914 (6) have given similar results. Most noteworthy of the investigations in this particular field have been those of Dobell and Laidlaw (7), Dobell, Laidlaw and Bishop (8), St. John (9), and Bonnin and Aretas (10). These authors, in agreement with Vedder, have shown by a variety of experimental methods that emetine or its salts have a direct amebacidal action which is effective in very high dilutions (1 to 1,000,000 to 1 to 5,000,000).

Due to its powerful emetic action the oral administration of emetine has been unsatisfactory. Rogers (11) advocated the subcutaneous administration of the hydrochloride salt to avoid the nausea and vomiting caused by preparations of emetine taken orally. There was an immediate widespread acceptance of this method of therapy and in general, the results have justified the continued use of the drug. Unfortunately, treatment with emetine parenterally is not without its dangers. Clinical experience, autopsy findings, and animal experimentation have shown emetine to be a toxic drug when administered by hypodermic injection, (12), (13), (14).

Because of the toxicity of subcutaneous emetine, its therapeutic dosage has been limited. Craig (15) states that the amount which can be safely given is, in the majority of cases, insufficient to permanently eradicate the parasite. Therefore, it is used as an adjuvant with other drugs such as chiniofon, carbarsone,

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TABLE 1  
1 grain daily for 12 days—total 12 grains

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTOSCOPIC	HB % SAHLI	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION					COMMENT		
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	Ameba culture	HB % sahl		Proctoscopic	
1	33	Amebic dysentery.* Other intestinal parasitic sites: <i>N. americanus</i> , <i>T. trichiuris</i> .	Diarrhea, mod. severe for one month, sensation of weakness, abdominal cramps and tenesmus. Heavy feeling in back.	Small ulcerations lower 1/3 of rectum	75	<i>E. hist.</i> trophozoites. Other parasites	Not done	Neg.	Before treatment. Normal limits. 3, 6, 11th days of treatment. Normal limits.	Symptoms subsided in 3 days. No amebae after 3rd day. Rectal ulcers healed on 11th day Rx.	No toxic symptoms. Gained 4 lbs. in weight.	7 mo.	Neg.	Neg.	Neg.	84	Neg.	No symptoms since original Rx.	
2	52	Amebic dysentery. Carrier of <i>E. typhosa</i> . Other intestinal parasitic sites: <i>E. coli</i> , <i>C. mesentericus</i> , <i>N. americanus</i> , have	Periodic attacks of diarrhea and abdominal pain for past year. 2 weeks prior to admission began to have	Normal findings	75	<i>E. hist.</i> trophozoites. Other parasites.	Not done	Several positive for <i>E. typhosa</i> .	Before Rx: Normal limits. 3, 6, 10th days of Rx: Normal limits.	Symptoms subsided in 3 days. No amebae after 4th day.	None	9 days	Cysts & trophozoites of <i>E. hist.</i>					Given another course of 12 grs. in 12 days. No amebae found after 2 days of Rx. Rechecked in 3 mos. and again in 5 mos. No symptoms in interim.	

[illegible]

TABLE 1—Continued

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTOSCOPIC	HB % SABI	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION					COMMENT		
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	Ameba culture	HB % sabb		Proctoscopic	
4	35	Amebic dysentery. Other intestinal parasitic sites: <i>E. nana</i> , <i>E. coli</i> , <i>D. fragilis</i> .	History of abdominal pains described as "gas pains" for past 5 years associated with tired feelings. For past 2 days has had severe cramps with 5-6 stools per day.	Small ulcerations throughout.	70	<i>E. hist.</i> trophozoites. Other parasites.	Not done	Neg.	Before Rx: Normal limits. 5, 9, 12th days of Rx: Normal limits.	No amebae after 2 days.	Vomited once. No nausea or abdominal cramps.	5 mo.	Neg.	Neg.	Neg.	78	Neg.	No symptoms.	
5	25	Amebic dysentery.	Bloody diarrhea and abdominal cramps for 9 mos. 2 to 12 stools	Rectal ulcerations severe, most numerous on inf. valve.	75	<i>E. hist.</i> trophozoites.	Not done	Neg.	1, 5, 12th days Rx: Normal limits.	No amebae after 4 days. No symptoms after 3rd day.	Vomited twice. No abdominal cramps or other symptoms.	1 mo. 2 mo. 3 mo.	Neg. Neg. Neg.	Neg. Neg.				Rechecked as outpatient only. No proctoscopic examination. No symptoms.	

6	23	Carrier of <i>E. hist.</i> Other intestinal parasites: <i>N. americanus</i> , <i>S. stercoralis</i> , <i>T. trichiuris</i> , <i>A. lumbricoides</i> .	per day. Cramps relieved by "passing gas." Diarrhea worse after liquor.	Normal findings	75	<i>E. hist.</i> cysts.	Positive for <i>E.</i> <i>hist.</i>	Neg.	Before and af- ter Rx: Normal limits.	No amebae after 3rd day.	None	4 mo.	Neg.	Neg.	Neg. 84	Neg.	No symp- toms.
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TABLE 1—Continued

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTOSCOPIC	HB % SATURI	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION					COMMENT	
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	Ameba culture	HB % satili	Proctoscopic	
7	56	Amebic dysentery.	Onset 2 weeks prior to admission of 6-10 stools per day with blood noted on one occasion, but denies abdominal cramps. Had dysentery in 1910 with similar symptoms. Was treated by "injections."	Rectal ulcerations severe, with almost entire rectum involved. Direct smear showed enormous number of <i>E. hist.</i> trophozoites.	75	<i>E. hist.</i> trophozoites.	Pos.	Neg.	Before Rx: Normal limits, 3, 6, 10th days of Rx: Normal limits.	No amebae seen by direct smear after 4 days. Culture for amebae negative following 6th day. Symptoms relieved on 3rd day.	None	3. mo.	Neg.	Neg.	Neg.	84	Neg.	No symptoms.

\* Terminology of War Department Tech. Bull. 759, May, 1945—*Amebic dysentery*: Cases of amebiasis with intestinal symptoms and abnormal stools which contain motile amebae. Carrier of *E. histolytica*: Cases in which there are no symptoms and cysts alone are found.



etc. It is still the drug of choice in the management of extra-intestinal amebiasis.

Since emetine has a powerful amebacidal action it is surprising that more attention has not been given to the possibilities of developing this drug in a form for oral use. A few attempts have been made to cover the drug with salol, or a keratin coating, in order to resist the action of the digestive juices and permit release of the drug lower in the bowel, or combining the salt with other drugs in an attempt to lessen the emetic properties. The currently available forms such as emetine bismuth iodide, emetine antimony iodide, etc., still cause salivation, nausea, and vomiting, and on the whole have not been successful.

In July, 1943, a small quantity of emetine hydrochloride in "enteric-sealed" tablets<sup>1</sup> was obtained. The tablets were designed to release their contents from 3 to 4 hours after ingestion and thus allow the drug to be freed in the lower bowel and avoid the irritating effects on the stomach.

#### PROCEDURE AND MATERIAL

This study is a report of the investigation of the first 20 patients in which the "enteric-sealed" oral preparation of emetine hydrochloride had been used for the treatment of intestinal amebiasis. Included in this group of patients are Latin Americans, British West Indians, and North Americans, of both sexes, and of age groups from 2 to 56 years. Each patient was proven to harbor *Endameba histolytica* before treatment was instituted, and all were under the complete care, as hospital patients, of the senior author (B. S.) during the course of treatment. The following routine was established:

(1) Daily stool examinations by one of us (C. J. or B. S.) for amebae. Smears following saline purges and culture methods were not used for these, but were used in the re-examination studies. Practically all cases, however, harbored other intestinal parasites, and were given purges and anthelmintics following the emetine treatment. The opportunity of using these stool specimens in searching for amebae, was not neglected.

(2) Daily culture of stools for micro-organisms.

(3) Daily urinalysis.

(4) Daily blood pressure reading.

(5) Complete blood count every third day.

(6) Electrocardiogram every third day, except on infants.

(7) Proctoscopic examination before and after treatment.

(8) Accurate count of number of stools passed per day.

(9) All individuals were examined on daily rounds for any signs of toxicity and closely questioned for any symptoms of vomiting, diarrhea, abdominal pains, malaise, or neuritides.

At the time this investigation began, no specific data were at hand relative to the amount of emetine absorption which might occur from the intestine. The therapeutic and the toxic dosage of the oral preparation were unknown.

<sup>1</sup> Emetine hydrochloride "Enseals" (Enteric-Sealed Tablets, Lilly). Each tablet containing  $\frac{1}{2}$  grain of the alkaloid.

TABLE 2  
2 grains daily for 5 days—total 10 grains

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTOSCOPIC	HB % Sahlb	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION					COMMENT	
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	Ameba culture	HB % Sahlb		Proctoscopic
8	25	Amebic dysentery, chronic. Other colicky abdominal pains for 1 year. Has lost 10 lbs. in weight in past 6 months.	Periodic attacks of diarrhea and colicky abdominal pains for 1 year. Has lost 10 lbs. in weight in past 6 months.	Normal findings.	75	<i>E. hist.</i> cysts. <i>E. coli</i> cysts.	Not done.	Neg.	Before and after Rx: Normal limits.	Negative for amebae in 3 days.	Vomited 3 times with slight temporary increase in no. of stools. No abdominal cramps. One pill passed in stool.	1 mo. 2 mo.	Neg. Neg.		Neg. Neg.			Rechecked as outpatient only. No attacks of diarrhea or cramps since treatment.
9	25	Amebic dysentery.	Periodic attacks of diarrhea for past 3 years. Sometimes constipated, has steadily lost weight.	Large ulcerations of rectum. Direct smear positive. 1 day after Rx: no evidence of ulcers.	80	<i>E. hist.</i> trophozoites.	Not done.	Neg.	1, 5th day of Rx: Sinus arrhythmia, otherwise normal. 2 days after Rx: Sinus arrhythmia, other	No amebae after 4 days. No further symptoms after 3rd day.	Vomited once. Lost 3½ lbs. during Rx.	Not obtained						

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10	13	Amoebic dysentery. Secondary amoebiasis. Sick-lemia, severe. Sick-lemia, mild. Other intestinal parasites: <i>N. americanus</i> , <i>A. lumbricoideus</i> , <i>T. trichiuris</i> , <i>S. stercoridis</i> , <i>E. coli</i> , <i>G. lamblia</i> .	Has had a bloody diarrhea for past 6 months. Father states child has shown a progressive listlessness and apathy and seems to be losing weight.	Entire rectum and sigmoid covered with enormous masses of <i>T. trichiuris</i> worms. No amoebic ulcerations noted.	40	<i>E. hist.</i> trophozoites. Other parasites.	Not done.	Neg.	1, 5th day of Rx: Sinus tachycardia otherwise normal. 2 days after Rx: Sinus tachycardia otherwise normal.	No amoebiasis after 4 days. Diarrhea stopped after 5th day.	None. Severe trichiurias infection. Sed. mto 74.	3 mo.	Neg.	Neg.	72	Trichiurias seen in rectum.	Marked improvement in blood picture and general health. No symptoms since treatment.
			Relieved by paregoric, but not stopped. Usually has 6 stools per day. The last attack has lasted for 15 days.							wise normal.							

TABLE 2—Continued

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTOSCOPIC	HB % SAHLI	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION						COMMENT
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	Ameba culture	HB % sahl	Proctoscopic	
11	19	Amebic dysentery, chronic. Anemia, secondary.	No symptoms other than gradual weight loss for past 3 years.	Normal	70	<i>E. hist.</i> trophozoites.	Not done.	Neg.	1, 5th day. Normal limits. 2 days after Rx: Normal limits.	No amebae after 4 days.	None	1 mo.	Neg.		Neg.			Rechecked as outpatient only.
12	41	Amebic dysentery. Other intestinal parasitic sites: <i>N. americanus</i> , <i>S. stercoridis</i> , <i>T. trichiuris</i> .	Abrupt onset one month ago of 7-10 loose liquid stools per day, associated with marked tenesmus.	Rectal ulceration severe. Direct smear positive.	80	<i>E. hist.</i> trophozoites. Other parasites.	Not done.	Neg.	First Rx: Normal limits. (3, 6th day) Second Rx: 1, 4th day; Normal limits.	Symptoms subsided in 3 days. No amebae after 3 days.	None Cysts of <i>E. hist.</i> found 6 days after 1st Rx. Re-treated.	6 days	<i>E. hist.</i> cysts.		<i>E. hist.</i>		2 days after 1st Rx: Ulcerations gone but several red-dish areas present. Direct smears negative. Proctoscopic negative. Hb. 4 days later normal appearing mucosa.	Given re-treatment of 2 grains daily for 6 days. No <i>E. hist.</i> after 4 days. Rechecked again in 1 month, and found positive for <i>E. hist.</i> , but denied any symptoms. Proctoscopic negative. Hb. 84%. Given re-treatment of 2 grains daily for 8 days.



TABLE 3  
Larger doses

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTOSCOPIC	HB % SABI	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION					COMMENT	
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	ameba culture	HB % sabli		Proctoscopic
14	45	Amoebic dysentery. Secondary anemia, severe. Pansinusitis, acute. Other intestinal parasitic sites: <i>N. americanus</i> , <i>S. Stercoraria</i> .	Acute diarrhea and abdominal pains for past 8 days. Has 0-8 liquid stools daily. No vomiting.	Several discrete ulcerations sprinkled throughout rectum and sigmoid. Mucosa pale.	40	<i>E. hist.</i> trophozoites. Other parasites.	Not done	Neg.	Before Rx: Normal limits. 3, 6th day. Normal	No amebae after 10 days.	Treatment: 1 gr. daily for 7 days, then 2 gr. daily for 4 days: 15 grains in 11 days. Diarrhea persisted. Large amount of inflammatory exudate in stool, culture negative. Given sulfanamide, with relief of diarrhea.	6 mo.	Neg.	Neg.	Neg.	82	Normal	No symptoms since original Rx. Marked improvement of anemia.
15	20	Carrier of <i>E. hist.</i> Other intestinal parasites found on routine para	No symptoms. Parasites found on routine	Neg.	80	<i>E. hist.</i> cysts. Other parasites.	Not done	Neg.	2, 7th day of Rx: Normal limits.	No amebae after 3 days.	Treatment: 1 gr. daily for 9 days, then 2 gr. daily for	4 mo.	Neg.	Neg.	Neg.	90	Normal	No symptoms since treatment.

10	30	Amoebic dysentery. Other intestinal parasites: <i>A. lumbricoides</i> . Secondary anemia, severe. Pregnancy.	stool examination.	Scattered discrete pinhead ulcers through-out rectum, below sup. valve.	42	<i>E. hist.</i> trophozoites. Other parasites.	Positive for <i>E. hist.</i>	Neg.	1, 5, 9, 12, 15, 19th days of Rx: Normal limits.	<i>E. hist.</i> trophozoites present in stools throughout course of Rx.	3 days: 15 grains in 12 days. None	3 mo.	Pos. <i>E. hist.</i>			54	Rectal ulcers present.	Has felt well since original Rx. 1 1/2 months pregnant. Given re-treatment of 2 grains daily for 8 days. No toxic symptoms. No <i>E. hist.</i> by smear or culture after 5 days.
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TABLE 4  
Infants and children

CASE NO.	AGE	DIAGNOSIS	SYMPTOMS	PROCTO-SCOPIC	HB %	STOOL EXAMINATION			E.C.G.	IMMEDIATE RESULT	TOXIC SYMPTOMS AND COMMENT	RE-EXAMINATION						COMMENT
						Direct smear	Ameba culture	Bacteria culture				Time since Rx	Direct smear	Saline purge	Ameba culture	HB %	Procto-scopic	
17	9½	Amebic dysentery. Other intestinal parasite: <i>T. trichiuris</i> .	Onset 2 weeks ago of 4-10 day but with no abdominal cramps. Never noticed blood in stools.	Normal	80	<i>E. hist.</i> trophozoites. Other parasites.	Positive for <i>E. hist.</i>	Neg.	Before Rx: Sinus arrhythmia. 1, 5th day of Rx: No change	No amebae after 3 days	Treatment: 1 grain twice daily for 6 days: (4 grains total). Vomited once.	Not obtained	Neg.	Neg.	Neg.	78	Normal	No symptoms
18	11	Carrier of <i>E. hist.</i> Other intestinal parasites: <i>N. americanus</i> , <i>E. coli</i> , <i>E. nana</i> , <i>G. lamblia</i> , <i>T. trichiuris</i> , <i>S. stercoralis</i> , <i>A. lumbricoides</i> , <i>T. hominis</i> . Secondary anemia.	Absolutely denies any symptoms!	Negative	65	<i>E. hist.</i> cysts only. Other parasites.	Not done	Neg.	Before Rx: Sinus arrhythmia. Normal limits. 7, 10th days of Rx: No change.	No. <i>E. hist.</i> after 4 days. Ro-1 mained positive for <i>E. coli</i> cysts.	Treatment: 1 grain twice daily for 12 days (total 8 grains). None.	3 mo.	Neg.	Neg.	Neg.	78	Normal	No symptoms



19	1½	Amebiodysentery. Other intestinal parasites: <i>G. lamblia</i>	Bloody diarrhea of 5 week duration associated with occasional rectal prolapse. Poor appetite, irritable and has lost weight.	Not done	70	<i>E. hist.</i> trophozoites. Other parasites.	Positive for <i>E. hist.</i>	Neg.	Not done	No amebae after 4 days.	Treatment: ½ grain daily for 9 days (total 3 grains). Given sulfanilamide at same time for six days. No toxic symptoms. Bloody diarrhea ceased after 3 days. No further prolapse.	3 mo.	Pos. <i>E. hist.</i>	<i>E. hist.</i>	54	Not done	Appetite for past month poor. "Occasional" diarrhea. Given treatment of ½ grain daily for 15 days (total 10 grains). Recheck in 3 mo. showed <i>E. coli</i> , <i>C. mami</i> , <i>G. lamblia</i> , but no <i>E. histolytica</i> .
20	1½	Amebiodysentery, chronic. Other intestinal parasites: <i>E. coli</i> , <i>E. intestinalis</i> , <i>C. mami</i> , <i>G. lamblia</i> , <i>T. hominis</i> . Secondary anemia.	Onset one week ago when, associated with mild fever. Has had 3 similar episodes in the past year. Appetite remains good.	Not done	60	<i>E. hist.</i> trophozoites. Other parasites.	Not done	Neg.	Not done	No amebae after 4 days.	Treatment: ½ grain daily for 9 days (total 3 grs.) None.	2 mo.	Neg.	Neg.	72	Not done	No symptoms

*Analysis of results following 1 grain daily for 12 days*

Inasmuch as one grain a day for 12 days had been used at Gorgas Hospital as the maximal dose in the parenteral method of administration, a similar amount was given by mouth in the first 7 cases. (See table 1). The patients received 1 tablet of  $\frac{1}{2}$  grain of emetine hydrochloride, orally, three times a day for 12 days.

No serious toxic effects were noted in these patients. The pulse rates, blood pressures, urinalyses, blood counts, electrocardiograms, all remained within normal limits. The blood picture in cases with anemia usually improved. One patient vomited once, and another vomited twice. The vomiting was abrupt and sudden, not accompanied by nausea or abdominal cramps. The drug was continued without any increase in this symptom. These isolated vomiting spells were unexplained until it was noted that one of the tablets in a bottle had lost part of its covering. No further vomiting occurred after discarding broken tablets.

A mild, non-bloody diarrhea of 3 to 5 stools per day occurred in a few cases, but no tenesmus or abdominal cramps were noted. One patient's (2) stools became entirely negative for amebae after 2 days of treatment but showed trophozoites and cysts nine days after the completion of treatment, and before being discharged from the hospital. This was the only immediate failure on this dosage. He was successfully given another course of one grain per day for 12 days, at an interval of 13 days from the original treatment. The stools became negative for amebae in 2 days. Re-examination in one month, and six months, showed no *Endameba histolytica*.

*Analysis of results following 2 grains daily for 6 days*

Encouraged by these results the drug dosage was doubled and the number of days halved. This dosage was used in 6 cases (see table 2). The patients thus received 2 tablets of  $\frac{1}{2}$  grain each, three times a day, for 6 days. No serious toxic reactions were noted in this series. Vomiting occurred in two cases (8), (9). Neither complained of abdominal cramps or nausea. There was one immediate failure in this series (12). *Endameba histolytica* cysts were found 6 days after completion of treatment, and he was immediately given a second course of the drug, which was successful, and no toxic symptoms were manifested. There was one delayed failure (13) in which *E. histolytica* was found by culture one month after treatment. There had been no symptoms in the interim and the patient had gained 5 pounds. When another similar course of emetine was given the parasites disappeared in 4 days.

*Analysis of results following larger doses*

Three cases were given varying dosages. (See table 3.) Case 16 was the only case in the entire series of twenty whose stools remained persistently positive for *E. histolytica* trophozoites. This patient was given 1 grain per day for 15 days, then 2 grains daily for 4 days, or a total of 23 grains, in 19 days, after which healing of the rectal lesions occurred, and there was complete relief of symptoms. She requested discharge for personal reasons but returned 3 months later. At this time amebic rectal ulcerations were again found and she was given treatment

of 2 grains daily for 8 days. The stools became negative by smear and culture in 5 days, and the rectal ulcerations disappeared.

### *Analysis of results in infants and children*

There were 4 infants and children in this series (see table 4). These were given much smaller doses than adults. There was one delayed failure in this group. This child (19), with severe amebic dysentery, was given 1 tablet ( $\frac{1}{3}$  grain) daily for 9 days, a total of 3 grains. Complete relief of symptoms was obtained, but a re-examination in 3 months demonstrated *E. histolytica* trophozoites. He was then given 2 tablets a day ( $\frac{2}{3}$  grain), for 15 days, a total of 10 grains, without manifesting toxic symptoms.

### *Results of re-examinations of patients*

We were able to re-examine 18 of the 20 cases in from one to seven months following the original treatment. Three of these were examined as outpatients, and only stool examinations were done, using direct smear and culture methods. The remaining 15 cases were re-admitted as hospital patients and subjected to complete studies. Three direct smears from each of several normally passed stools were first studied. If these were negative, a saline purged stool was obtained, and three smears from each of several specimens of this stool were carefully examined microscopically. Daily cultures, at least one of which was from the saline purged stool, were taken on St. Johns medium as described in Craig (15). All patients except the infants, had a proctoscopic examination. Any case in which either cysts or trophozoites of *Endameba histolytica* were found, was considered a failure, disregarding the presence or absence of symptoms, or the time since the original treatment.

It is interesting to note that although 5 of these 20 patients were not cured from a parasitological standpoint, 4 of them became symptom-free, and remained so, since the original treatment. One case (19) had a history of "occasional diarrhea, poor appetite" for one month prior to admission for re-examination, which was done 3 months following the original treatment.

### DISCUSSION

The present series of cases is small but some preliminary conclusions can be drawn from the results obtained. Oral emetine therapy (with "enteric-sealed" tablets) for intestinal amebiasis deserves further study. Reed (16) states that "emetine is a powerful, dangerous, and valuable remedy whose complete action is not known." The results obtained in our preliminary study indicate that when used as described in this series, it is not dangerous. When given parenterally, or in a form which permits rapid absorption from the stomach or upper intestinal tract, it may be a toxic substance. In our patients, when the drug was given in such a form that it theoretically reached the distal portion of the small intestine or the colon before being liberated, no serious toxic reactions were noted.

The presence of the alkaloid in the upper intestinal tract is usually attended by nausea and vomiting. A few of the patients in this series experienced vomiting. This symptom occurred only once or twice during the course of treatment. It

was probably caused by the use of chipped tablets, which allowed the emetine to be released in the stomach or upper intestinal tract. The mild diarrhea which appeared in some cases during the treatment was not considered as an indication for withdrawing the drug.

#### SUMMARY

In a preliminary study of 20 cases of intestinal amebiasis due to *Endameba histolytica*, including both acute and chronic forms, treated with emetine hydrochloride enteric-sealed tablets orally, encouraging results were obtained in 15 patients in a short period of time. These patients have been observed over periods of time ranging from one to seven months. None of the usual serious toxic reactions associated with the parenteral administration of emetine were noted. Results were judged on the basis of clinical improvement, healing of the bowel as observed by proctoscopic examination, and the disappearance of the amebae in microscopic studies and cultures of the stools. No recommendations as to the optimum dosage for the treatment of intestinal amebiasis with the oral emetine preparation used in this study can be given at this time. Further evaluation of this preparation is now in progress.

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# PRELIMINARY REPORT ON THE EVALUATION OF PENICILLIN IN THE TREATMENT OF YAWS\*

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## REVIEW OF LITERATURE

The recent development of penicillin therapy in early syphilis suggested the possibility that this drug might play a similar rôle in the treatment of yaws, a closely related spirochaetal disease. A few reports in the literature tend to bear this out. Whitehill and Austrian (1944, 1945) reported the treatment of 41 cases of primary and secondary yaws among Fijians. They used total doses of approximately 500,000 Oxford Units of penicillin in aqueous solution, giving 15,000 O. U. intramuscularly every four hours for five or six days. Dark-field examinations became negative in 16 hours after treatment, and most lesions healed in one week. No cases were rendered permanently sero-negative with the Kahn test within 20 weeks, the extent of their follow-up period at the time of publication. Lofgren (1944) reported the treatment of a white sailor who had contracted yaws on American Samoa. He used a total dose of 1,500,000 O. U. of penicillin in aqueous solution over 12 days. The dark-field examination became negative in 18 hours. All secondary lesions healed in five days and the primary lesion, which had been ulcerated, in 13 days. The Kahn test became negative five weeks after treatment. da Cunha, Leao, Guimaras and Cardoso (1944) treated seven cases of yaws in Brazil with total doses of 9,600-52,000 O. U. of penicillin and obtained clinical cures in 12 to 44 days. They stated that serologic reactions (Wasser-

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mann test) became negative 60 days after treatment in all cases. da Cunha and Leao (1945) treated an additional five cases in Brazil with similar results. Findlay, Hill and Macpherson (1944) reported the treatment of 24 cases of yaws in children in Africa with 50,000-100,000 O. U. of penicillin in aqueous solution over a period of 12 to 24 hours. Clinical cures were obtained in six to seven days. Reversal of the Kahn test in two of the primary cases was attained in seven days. In two of the secondary cases the Kahn test remained positive after six weeks. Tompsett and Kauer (1945) reported five cases of yaws among Melanesians on New Guinea treated with a total dose of 250,000-400,000 O. U. of penicillin in aqueous solution over a period of two to four days. The dark-field examinations became negative in 24 hours, and the lesion healed in one to three weeks. No serologic tests were done after treatment.

TABLE 1  
*Age distribution of and total amount of penicillin given to yaws patients*

AGE GROUPS	5 YEARS AND UNDER	6-12 YEARS	13-16 YEARS	17 YEARS AND OVER	TOTAL
Series A.....	13	60	41	86	200
Total dosage penicillin in 4 days.	1,200,000 O. U.	1,200,000 O. U.	1,200,000 O. U.	1,200,000 O. U.	
Series B.....	0	74	27	50	151
Total dosage penicillin in 2 days.		600,000 O. U.	900,000 O. U.	1,200,000 O. U.	
Series C.....	0	56	35	58	149
Total dosage penicillin in 1 day..		600,000 O. U.	900,000 O. U.	1,200,000 O. U.	
Total Number Patients.....	13	190	103	194	500

The foregoing survey of the literature would seem to indicate that the use of penicillin in the treatment of yaws invariably resulted in complete clinical cure, even in relatively small doses. On the other hand, only a few of the cases were observed to attain sero-negativity with the Kahn or Wassermann test after treatment. Therefore, the value of penicillin in the treatment of yaws has not been definitely established.

#### MATERIALS AND METHODS

The present paper constitutes a preliminary report on the treatment of 500 patients with primary and secondary yaws infections in Haiti with penicillin. The patients were divided into three series as follows:

Series A. Two-hundred patients were hospitalized and given a total of 1,200,000 O. U. of penicillin sodium in aqueous solution each over a period of four days. They received 30 intramuscular injections of 40,000 O. U. each, one every three hours day and night. All patients received the same total dose, regardless of age. The age distribution of the patients is given in table 1.

Series B. One hundred and fifty-one patients were treated on a two-day ambulatory basis with penicillin calcium in peanut oil with 4.8 per cent beeswax by weight (300,000 O. U. per cc.). The dosage was graded down for children on the basis of age and approximate weight. The age distribution of the patients and the total amount of penicillin administered are given in table 1. Children six to 12 years of age received 600,000 O. U.; patients from 13 to 16 years of age, 900,000 O. U.; and those 17 years old and over, 1,200,000 O. U. The drug was given by intramuscular injections in divided doses 24 hours apart.

Series C. One hundred and forty-nine additional patients were treated on a one-day ambulatory basis with penicillin calcium in oil with beeswax. The dosage was again graded down for children. The age distribution of the patients and the total amount of penicillin administered are given in table 1. Children six to 12 years of age received 600,000 O. U.; patients from 13 to 16 years of age, 900,000 O. U.; and those 17 years old and over, 1,200,000 O. U. The drug was given by intramuscular injections in divided doses 10 to 12 hours apart.

Special medical histories were taken on each patient prior to treatment. Emphasis was placed on exact location of home, previous history of yaws, age of onset, location and duration of primary and secondary lesions, previous treatment, and presence of yaws in other members of the family. If other members of the family were infected the patient was urged to bring them in for treatment so that they would not serve as foci of reinfection. Physical examination was limited to close observation of the skin and muco-cutaneous borders. Prior to treatment 20 cc. of blood was taken from each patient for a battery of serologic tests (Kline Diagnostic, Kline Exclusion, Slide-Flocculation Test employing cardio-lipin antigen, Mazzini, Quantitative Kahn and Quantitative Kolmer). The blood was permitted to clot and was refrigerated in most instances for variable lengths of time up to one week. It was then centrifuged, and the serum was drawn off. The serum was placed in vials containing powdered merthiolate (1 mg. per cc. of serum) and shipped by air to the Division of Serology, Army Medical School, Army Medical Center, Washington, D. C. The specimens arrived in excellent condition. Less than one per cent were unsatisfactory for testing.

The combination of clinical and serologic examinations was considered to be sufficient to establish the diagnosis of yaws. Further confirmation of diagnosis was obtained on one group of 26 patients in Series A on whom dark-field microscope examinations of serum from lesions were made. All were found positive for spirochaetes.

Considerable difficulty was and is being experienced in getting the patients to return for follow-up clinical observations and blood for serologic tests. Upon discharge from the hospital or clinic each patient was given an individual talk explaining the importance of follow-up observations. In addition, each patient was given a small printed card with his or her name, number, date and place of return. With regard to the latter, the patients were instructed to return to one of the yaws clinics or to the hospital, whichever was most convenient. Since most patients had to travel long distances by foot each of them was given 10 cents (gold) for the purchase of food. When patients did not return on their scheduled

day, doctors and aides were sent out on horse-back from the nearest clinic to their homes. If the delinquent patients could not be located a letter was sent to the Chief of Section in which they lived asking his cooperation in getting the patients to a clinic. This system has been fairly successful, as indicated by the following figures for follow-up observations.

Series A.....	1 month	82.5%
	2 months	79.0%
	3 months	72.0%
	4 months	68.5%
	5 months	78.0%
	6 months	84.5%
Series B.....	3 months	59.6%
Series C.....	3 months	65.8%

Monthly follow-up observations will be continued on patients in Series A for a minimum of one year. Patients in Series B and C will be followed at three months intervals over the same period.

#### RESULTS OF TREATMENT

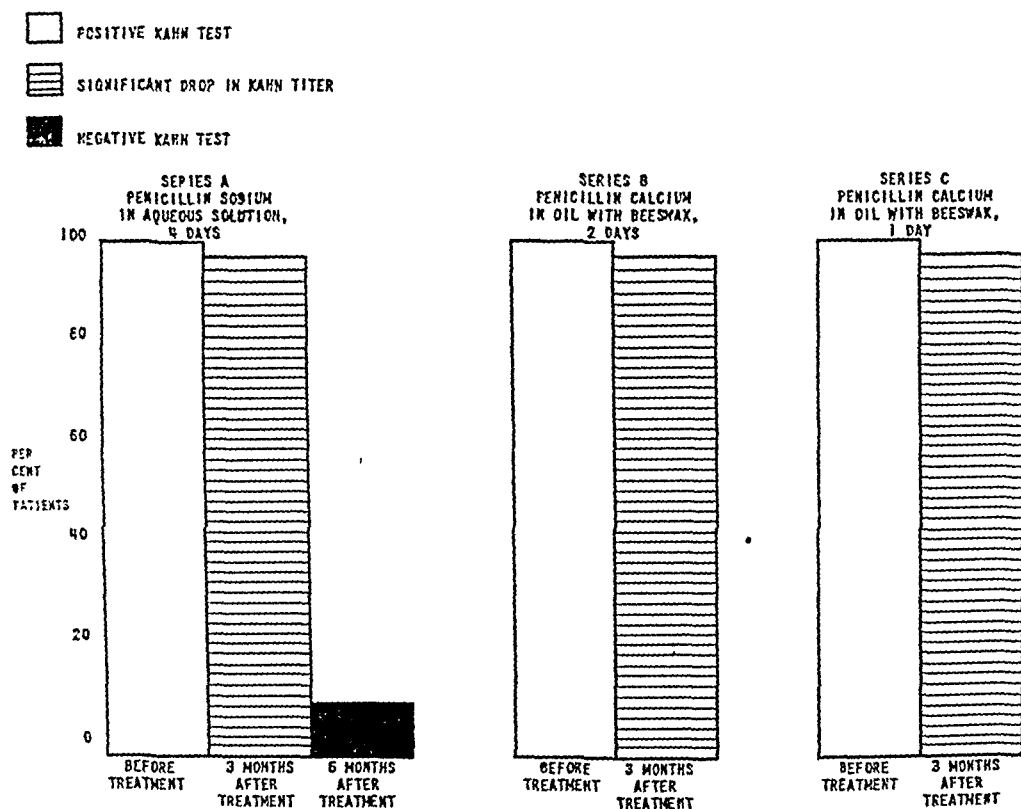
The clinical improvement of the treated patients in Series A was rapid and remarkable. Joint pains disappeared in 24 to 48 hours; plantar and palmar "crab" lesions became painless in 48 to 72 hours. Both primary and secondary lesions began to dry up in 24 hours. Epithelium grew in from the periphery and completely covered most lesions in three or four days. The great majority of patients who returned for observation one month after treatment showed complete healing of all lesions. A few ulcerated primary lesions with secondary bacterial infections were still draining pus at this time. Most of these healed spontaneously between the first and second month after treatment. Since patients in Series B and C were treated on an ambulatory basis, it was not possible to follow their immediate clinical courses. However, when these patients were seen three months after treatment, their first follow-up period, they showed complete healing of all lesions in most instances. Three of the patients had ulcerated primary lesions which were still draining pus.

Only 10 of the 169 patients in Series A followed for six months developed suspicious skin lesions after treatment. Whenever possible dark-field examinations were made of the lesions. The majority of the lesions were diagnosed as pyoderma. No primary lesions indicating re-infection were observed. Two patients developed painful dry plantar lesions which were tentatively diagnosed as "crab" lesions, indicating relapses. Dark-field examinations were not done on these lesions.

One patient in Series B developed painful dry plantar lesions three months after treatment. Here again a tentative diagnosis of relapse was made. One other patient in Series B developed a large lesion resembling typical primary yaws three months after treatment. Dark-field examination of this lesion was negative. The possibility that this may be a case of re-infection is being considered. Further observations must be made before a conception of relapse and re-infection rates can be formulated.



With regard to serologic observations, only three of the 500 patients examined were negative with the Kahn test prior to treatment. Of these, two showed some evidence of sero-positivity with one or more of the other five tests done. Three months after treatment 98 per cent of the patients in Series A showed definite evidence of reduction in serologic titer (Graph 1). Most of the cases showed a four-fold reduction in titer at this time. For example, if the degree of positivity was 128 Kahn units prior to the institution of therapy, it had dropped to 32 units at the end of three months. At the end of six months only 17 patients, or 10 per cent, in Series A had attained sero-negativity with the Kahn test. With very few exceptions the remaining patients showed a further slight reduction in sero-



GRAPH 1. SEROLOGIC REACTIONS OF PATIENTS IN SERIES A, B AND C BEFORE AND AFTER TREATMENT WITH PENICILLIN

logic titer as compared with their status at three months post-treatment. Patients in Series B and C showed a similar four-fold reduction in serologic titer at the end of three months. Only one patient had attained sero-negativity with the Kahn test at this time.

The other tests employed in the sero-diagnostic battery were somewhat more sensitive than the Kahn and gave higher titered reactions, but the general pattern and the amount of serologic improvement was similar in all tests. With reference to the four patients who developed lesions suggestive of clinical relapse or re-infection after treatment, nothing was found in the serologic pattern of these patients that differed from the other patients in the three Series. None of them

showed a serologic relapse prior to the development of cutaneous manifestations as has been observed in patients who have received penicillin therapy for syphilis. In the latter cases there has usually been a serologic relapse evident at about one month prior to the clinical relapse. The serologic data available at present does not lend support to the tentative clinical diagnosis of relapse in the cases under consideration.

#### PATHOLOGIC STUDIES

Dark-field examinations were made and biopsies taken on 10 patients in Series A with secondary lesions at two-hour intervals after treatment was instituted. The dark-field examinations became negative in eight to 12 hours, by which time the patients had received 120,000–160,000 O. U. of penicillin. Sections made from the biopsy material stained by the Warthens-Starry technique showed numerous spirochaetes up to 12 hours. Subsequently only occasional isolated ones were found up to 22 hours and none thereafter.

Histologic sections of secondary lesions taken prior to treatment showed the crusts and epidermis to be densely infiltrated with polymorphonuclear leukocytes. There were large numbers of spirochaetes in the epidermis and a few in the subepidermal papillary layer. The corium showed heavy infiltration with plasma cells and scattered foci of eosinophiles. The papillary layer was edematous. Twenty-four to 48 hours following treatment the crust became smaller, the process changing from parakeratosis to hyperkeratosis. The edema in the papillary layer gradually diminished and became replaced by collagen. These changes were nearly complete by the fifth day when treatment was terminated. However, the dense infiltration of plasma cells and eosinophiles in the corium persisted up to this time.

#### TOXIC REACTIONS

No severe toxic reactions were encountered. Approximately one-half of the patients in Series A had a rise in temperature to 100°–104°F. two to eight hours after treatment was started. All temperatures gradually returned to normal in 10 to 12 hours. Approximately one-fifth of the patients showed a brief secondary elevation of temperature on the third, fourth and fifth days of treatment. This febrile type of Herxheimer reaction would seem to indicate that penicillin had killed large numbers of spirochaetes. Temperatures were not taken on patients in Series B and C.

#### DISCUSSION

Although the clinical response to penicillin therapy was excellent and comparable in the three Series of yaws patients and comparable to that observed in a group of patients with early active syphilis, the serologic response was strikingly different. Whereas one would expect a serologic reversal of 70 to 80 per cent<sup>5</sup>

<sup>5</sup> Dr. J. E. Moore, personal communication, Penicillin Panel of the Subcommittee on Venereal Diseases of the National Research Council.

at the end of six months in a group of patients with early syphilis who had received a similar amount of penicillin therapy, it should be pointed out that only 10 per cent of the patients in Series A attained sero-negativity in the same period of time. Indications at the present time are that only a few more will turn sero-negative at the end of 12 months. In this connection it is interesting to note that Chambers (1938, 1944 a and b) reported that 25 per cent of 411 yaws patients treated with four to six weekly injections of neoarsphenamine turned negative with the Wassermann test six months after treatment, 44 per cent 12 months after treatment, 59 per cent 18 months after treatment, and 68.4 per cent 24 months after treatment. He reported similar results on 143 cases treated with four to six weekly injections of bismuth.

From Table 1 it will be seen that 73 of the patients in Series A were children under 12 years of age. These patients received the same amount of penicillin as the adults, namely 1,200,000 O. U. over four days. It is significant that although the majority of these children received as much as five times more penicillin in proportion to body weight than adults, the serologic response was in no way different from that of the adults. This would seem to indicate that the amount of penicillin beyond a certain minimum was not the essential factor in producing serologic cures.

It is possible that some of the patients may have had false positive serologic tests due to malaria and true positives due to concomitant syphilitic infection. The former seems unlikely since experience has indicated that false positive Kahn tests in patients with malaria persist only about four weeks after a clinical paroxysm. None of the patients had acute malaria at the time of treatment. Furthermore, there was very little fluctuation of the serologic titers in these patients as would have probably been the case if many false positive reactions had occurred. As regards syphilis, care was taken to exclude cases in which there was any question of the diagnosis of yaws clinically. However, it is likely that some errors in diagnosis were made. Fortunately, the incidence of syphilis in rural Haiti from which the great majority of the patients were drawn is low, being estimated by local authorities at about five per cent.

#### SUMMARY

In summary it may be said that penicillin holds definite promise as a therapeutic agent in yaws. Its use in doses of 1,200,000 O. U. in adults and proportionate doses for children given over periods of four, two and one days respectively resulted in rapid clinical cures. The serologic response was not as striking as the clinical, but until more follow-up figures are obtained a full evaluation is not possible. The oil-beeswax preparation of penicillin made it possible to treat patients on an ambulatory basis over one and two days with the same dose of penicillin given in aqueous solution to hospitalized patients over four days. The immediate clinical and serologic results obtained with penicillin in oil with beeswax appeared to be as good as those of the aqueous solution. The development of a successful one-day treatment schedule would be of great practical value in a country such as Haiti where large numbers of patients must be treated on an

ambulatory basis in rural clinics. A report on 12 months follow-up observations will be made at a later date.

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# A CASE IN WHICH EGGS OF SCHISTOSOMA JAPONICUM WERE DEMONSTRATED IN MULTIPLE SKIN LESIONS

HARRIS M. FISHBON<sup>1</sup>

The individual in this case, a Sergeant in the U. S. Army, was stationed on the island of Leyte in the Philippines from about November 25, 1944 to December 10, 1944. While there, he bathed in a slow-running stream. Snails were noted along the banks and native villages bordered the river.

About December 16 he noted angioneurotic swellings which appeared first about both eyes and then involved the entire face. This lasted three days and was associated with chills and fever. The temperature ranged from 100° to 103°F. The fever lasted for two weeks and was followed by a dry nocturnal cough which persisted for three weeks. With the pyrexia, the patient experienced weakness and anorexia.

At the same time that the cough appeared, about January 1, 1945, grouped pruritic papules developed on the right subchondral wall. A similar small cluster developed on the right side of the scrotal sac, but disappeared after four days. The rash began extending slowly toward the left abdominal wall, axilla and left side of the back, seemingly in an intercostal distribution. The lesions were infiltrative, shotty papules and tended to be grouped.

The individual was hospitalized on February 4, as several cases of schistosomiasis had been found in his organization. A positive stool examination for *Schistosoma japonicum* ova was obtained on February 7, 1945. Physical examination on February 14 revealed nothing significant except for the dermatitis. X-ray examination of the chest revealed no abnormalities. The WBC count was 8400, with 15 per cent eosinophils. The sedimentation rate was 19 mm. per hour.

The rash was herpetiform only in distribution and grouping, and was found in the region of the 8th intercostal space. The lesions were not vesicular. On the anterior abdominal wall were single hard, shotty papules, with a red areola and white fibrotic center, but the characteristic lesion was that of grouped or coalesced infiltrative papules. These were probably older lesions. The back showed lesions of a similar stage. Of these, there were fewer of the white fibrotic type and more of the early reddened grouped infiltrative papules, with an occasional one showing a minute purulent center (figures 1 to 4). The few papules having a pustular center were aspirated. Typical ova of *Schistosoma japonicum*, some showing viable miracidia and others in various stages of degeneration, were found in the purulent material. The clinically active lesions contained more of the viable ova than did the less active sites.

## PATHOLOGICAL REPORTS

(Provided by Major Joseph H. Bragdon, MC)

*Biopsy of abdominal wall:* (15 February 1945). "The specimen consists of an elliptical fragment of skin 2.5 cm. long and up to 1.5 cm. wide. The epithelium

<sup>1</sup> MAJOR, M.C., A.U.S.

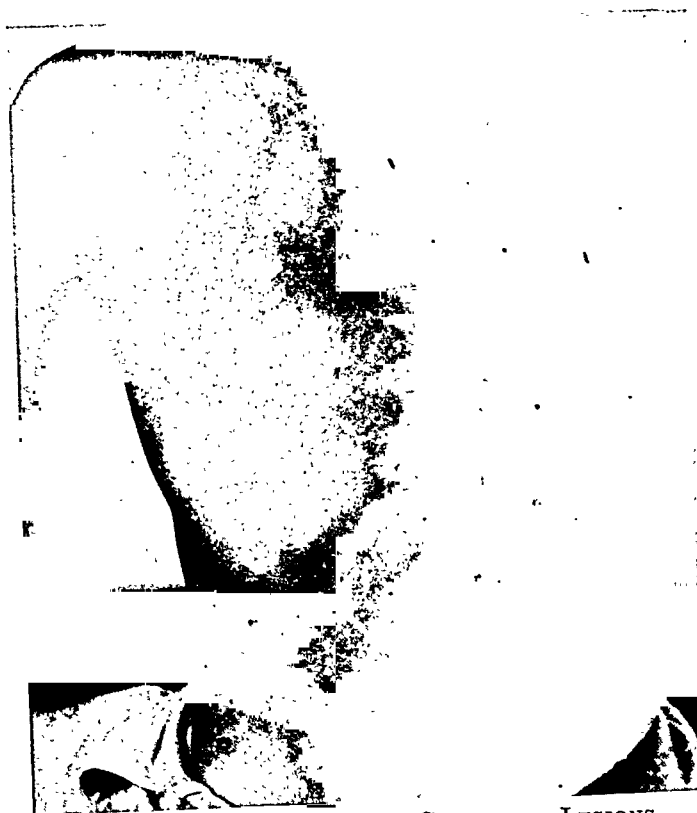


FIG. 1. LEFT BACK, SHOWING CUTANEOUS LESIONS



FIG. 2. LEFT ABDOMINAL WALL, SHOWING LESIONS



FIG. 3. LEFT BACK, SHOWING LESIONS



FIG. 4. LEFT BACK, SHOWING CUTANEOUS LESIONS

is everywhere intact, but there is a central papular elevation 6-7 mm. in diameter. The corium underlying this area appears firm and thickened. Microscopic sections taken at intervals through the block show scattered foci of inflammation in the corium. In each such area there are schistosoma ova. Most of the ova are markedly distorted and for the most part the shells remain empty. No evidence of an adult fluke is found. The ova are all surrounded by necrotic cellular debris. The inflammatory reaction is characterized by necrosis,

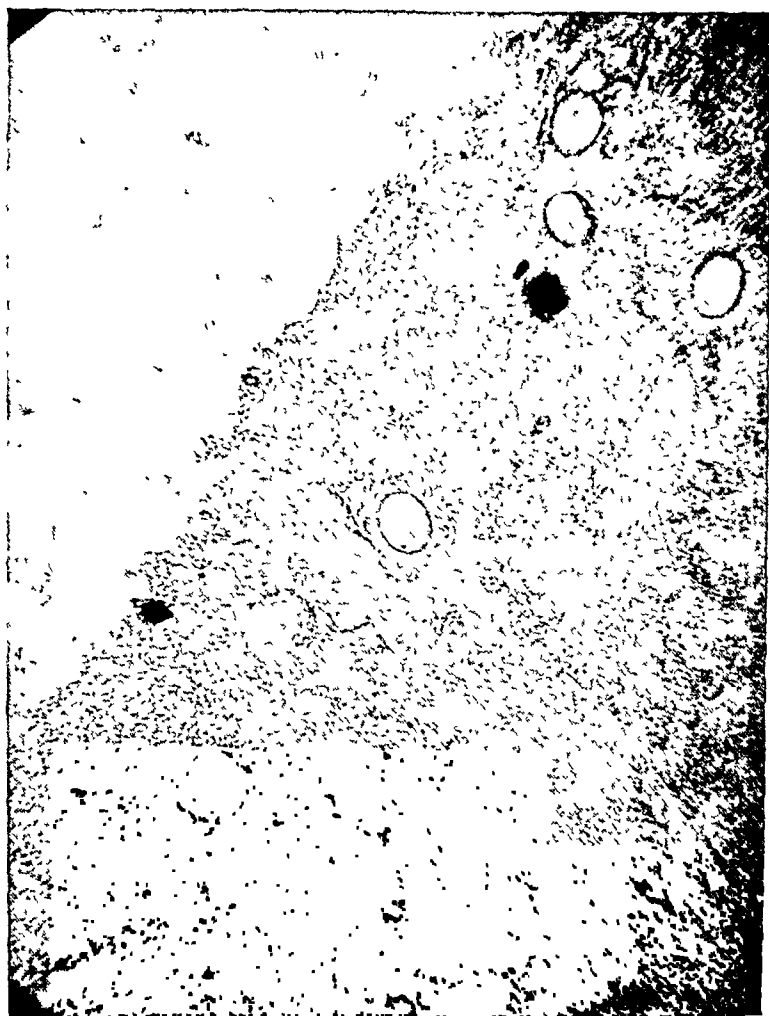


FIG. 5. OVA IN MATERIAL ASPIRATED FROM A PUSTULAR LESION

by a rather heavy infiltration of polymorphonuclears, lymphocytes, histiocytes, and eosinophils. Fibroblastic proliferation is not a prominent feature. In no site can any relationship to a blood vessel be established.

*Biopsy of back:* (8 March 1945). "Microscopic sections show much the same picture as that previously described. The ova are, however, much better preserved and the contained miracidia are well stained. (In this case an effort was made to biopsy a fresh lesion, and the tissue was fixed in formalin rather



than Zenker's fluid, as in the first case.) The histology differs in no essential way from that described above, except that the eosinophilic infiltration is more prominent."

#### Course

A 40 cc. course of Fuadin was started February 17 and completed March 3. During the course of treatment new lesions developed, with spreading to the



FIG. 6. BIOPSY SECTION SHOWING THREE OVA

right upper back. On March 13, ten days after completion of this course, the last new skin lesion containing ova with active miracidia was found. The WBC count was 10250, with 10 per cent eosinophils. The sedimentation rate was 14 mm. per hour. Stools were positive for *S. japonicum* ova.

A second course of Fuadin was given from March 23 to April 5. On the last observation of this patient (April 19), the skin lesions appeared as small white, flat, fibrotic papules. Physical examination revealed no abnormal findings.

Neither liver nor spleen was palpable. Proctoscopy was negative for schistosome nodules. The WBC was 9850, with 9 per cent eosinophils. The sedimentation rate was 13 mm. per hour. Stools remained positive for viable *Schistosoma japonicum* ova.

#### COMMENT

*Schistosoma japonicum* has a predilection for the portal system. In a small percentage of cases observed, diffuse lung infiltration was noted in X-rays taken



FIG. 7. MEDIUM POWER VIEW OF TISSUE SECTION SHOWING *S. JAPONICUM* OVUM, WITH SURROUNDING REACTION

during the period of cough which lasted from four to six weeks. Cerebral involvement has likewise been observed. Though embolic ova have been noted in cerebral and pulmonary vascular systems, no *Schistosoma japonicum* adult worms have been demonstrated in these vessels in man. However, Koppisch (1) has demonstrated the adult fluke in the pulmonary vessels in man in schistosomiasis mansoni.

This case presents manifestations of involvement of the peripheral circulation

as well as that of the portal. It is possible that a distant adult worm could produce such embolic ova skin lesions. However, considering the localized distribution and progress of the dermatitis, it is also possible that an adult fluke may have been situated in an intercostal vessel. In 1940 Garcia, Navarro and Bautista (2) reported a case in which eggs of *S. japonicum* were demonstrated in a skin biopsy from a child suffering from a chronic ulcer of the leg. Dysenteric



FIG. 8. HIGH POWER VIEW OF A TISSUE SECTION, SHOWING AN OVUM CONTAINING A MIRACIDIUM

symptoms were present and eggs of the parasite were observed in the child's stool specimen.

#### CONCLUSION

A case in which eggs of *Schistosoma japonicum* were demonstrated in multiple skin lesions is reported.

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# THE TREATMENT OF SCHISTOSOMIASIS MANSONI WITH UREA STIBAMINE (SQUIBB)\*

## PRELIMINARY REPORT

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PRATT

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Encouraged by the recent results<sup>1</sup> obtained on treating human filariasis with intensive doses of pentavalent antimony compounds, the writers have been using urea stibamine (Squibb) in the treatment of fourteen cases of schistosomiasis mansoni. Urea stibamine is a buff-colored powder containing approximately 35 per cent of pentavalent antimony; the drug is fairly soluble in water.

In order that effects of the drug could be better evaluated, only patients who were passing large numbers of live schistosome ova were selected for treatment.

All of them were hospitalized and subjected to a physical examination. Daily records of temperatures, pulses and respirations were kept, together with complete blood counts and urinalysis made every three or four days.

First, a small dose of 50 to 75 mg. was administered so as to determine any susceptibility to the drug; thereafter, three daily doses were given on an empty stomach at 8 a.m., 11 a.m., and 4 p.m.; all injections were intravenous. The daily total dosage was gradually increased until the maximum tolerated amount was being given. Toxic reactions were carefully recorded.

The selected group, which included negroes and whites, was made up of 7 male and 7 female Puerto Ricans. Their ages ranged from 12 to 36 years, with an average of 26 years for the men and 21 for the women. The men averaged a weight of 128 pounds and the women 96, yet half of them could be considered under weight for their ages. Their physical examinations revealed carious teeth and fungus infections of the feet, from a mild to a pronounced degree. None of them showed hepatosplenomegaly; only one third had symptoms referable to the schistosomiasis infection. These symptoms, were apparent in an intermittent diarrhea and tenesmus with or without, melena. Every patient had lived all, or part of his life in a known endemic schistosomiasis area.

Stool examinations were made daily before and during treatment and on as many daily specimens as possible for each follow-up examination. The technique

\* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the School of Tropical Medicine.

Read at the Annual Meeting of the American Society of Tropical Medicine at Cincinnati, Ohio, November 15th, 1945.

<sup>1</sup> Chemotherapy of Human Filariasis by the Administration of Neostibosan, J. T. Culbertson, H. M. Rose, José Oliver-González. *Am. J. Trop. Med.*, Vol. 25, No. 3, 271-274, May, 1945.

used for the quantitative determination of eggs in feces has been described previously.<sup>2</sup> The number of alive and dead schistosome eggs were recorded.

### RESULTS

The amount of drug tolerated varied from 3.400 to 10.125 g. The smallest dosage was given to a 12-year old white girl, weighing 73 pounds, who never tolerated more than 150 mg. and always showed symptoms of nausea, vomiting, facial edema, and palpitations. The largest amount, administered throughout a period of 18 days, was received by a 29-year old white male who weighed 131 pounds. On an average, the men tolerated 7.180 g., given throughout 16 days of hospitalization, whereas the women tolerated 6.690 throughout 13 hospital days.

TABLE 1

*Number of schistosome eggs in patients treated with urea stibamine (Squibb)*

PATIENT'S HOSPITAL NUMBER	TOTAL AMOUNT OF DRUG ADMINIS- TERED	EGGS PER CC.											
		Before treatment		End of treatment		Days after treatment							
						30		60		90		120	
		Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	<i>grams</i>												
A-7046	10.125	125	175	0	0	0	0	0	0	0	0	0	0
A-6560	7.305	225	25	0	0			0	0	0	0	0	0
A-6718	6.775	175	100	0	0	0	0	0	0	0	0	0	0
A-7047	4.325	325	200	400	200	0	0	0	0	0	0	0	0
A-6795	3.400	275	175	300	125	450	500	225	100	Patient Discarded			
A-7083	9.385	1375	850	450	300			0	0	0	0	0	0
A-7063	7.600	500	500	250	325	0	0			0	0		
A-6781	6.825	150	200	50	50	0	0	0	0	0	0	0	0
A-7223	6.495	150	50	50	50	0	0	0	0	0	0	0	0
A-7221	6.400	150	400	25	75	0	0	0	0	0	0	0	0
A-7224	5.250	700	800	100	200	0	0	0	0	0	0	0	0
A-7225	7.100	300	2000	175	1525	0	0	0	0	0	0	0	0
A-7244	5.010	775	450	Died									
A-7222	6.125	250	450	50	150	0	0	0	0			0	0

Toxic reactions to the drug which were observed in all patients may be classified as immediate or delayed. Immediate reactions were those that occurred within the first 15 minutes after administration of the drug and consisted of flushing, facial edema, hoarseness, dyspnea, tachycardia, abdominal pain, nausea, vomiting, profuse sweating, bradycardia, pallor, poor pulse volume, and a semi-shock like condition. These reactions usually appeared on the third to fifth day of treatment and persisted, more or less, throughout its course. It was especially true of the febrile response, anorexia, abdominal pain, mental depression, and tiredness. Delayed symptoms were generally the mildest, but if they persisted or produced definite discomfort, the drug was reduced or suspended, as necessary.

<sup>2</sup> Treatment of *Schistosomiasis mansoni* with Sodium Thiomalate (Anthiomaline), F. Hernández-Morales, Ramón M. Suárez, C. Kreiss Pratt. In press.

One death occurred in the women on the 9th day of treatment when a total of 5.03 g. of the drug had been given due to acute drug sensitivity.

Four patients gave persistent positive Kahn reactions. Hanger's liver function test was positive, from 1+ to 3+, in 10 out of the fourteen patients. All patients but one were infected with one or more of the following parasites: *Trichuris trichiura*, Hookworm, *Endamoeba coli*, *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Giardia lamblia* and *Iodamoeba williamsi*.

In 9 cases, there was a transitory fall in the total WBC to about 3,000. Twelve showed on eosinophilia that averaged a 9 per cent rise above the initial count; the highest count reached 36 per cent. Urinalysis revealed that, out of 14 cases, 4 showed traces of albumin and casts during the course of therapy; these patients had had essentially negative urines prior to treatment. An increase in the flocculation of the cephalin-cholesterol mixture was observed in most of them.

The effect of treatment on the schistosome infections as determined by the number of alive and dead schistosome ova in stools may be seen in table 1. At the end of treatment 10 patients still showed ova in the stool while 3 had negative stools. Examinations done one, two, three and four months later revealed that 12 out of the 13 remaining patients had stools negative for ova at every examination. Patient number A-6795 who was still passing ova 60 days after treatment had received only 3.40 g. of the drug. This case was then eliminated from the series, because the patient subsequently demanded further treatment and another drug was administered.

#### DISCUSSION AND SUMMARY

Fourteen patients infected with schistosomiasis mansoni were treated with urea stibamine (Squibb). The drug was administered intravenously; the total amount given varied from 3.40 to 10.125 g.

Twelve out of 14 patients had stools negative for ova of *S. mansoni* when examined on the 30th day after the end of treatment and have remained negative thereafter for an additional period of 90 days.

One patient with the stools positive 60 days after treatment, had received the smallest amount of drug. The presence of ova in the stools of this patient may be attributed to the insufficient amount of drug administered.

Another patient died during hospitalization as a result of treatment. Toxic reactions to the drug occurred frequently among all patients and consisted of flushing of face, facial edema, hoarseness, dyspnea, tachycardia, abdominal pain, nausea, vomiting, congestion of conjunctival vessels, headache, weakness, loss of appetite, fever, decrease in white cell count, and albuminuria.

Although the patients have had stools negative for ova of *S. mansoni* during a period of 4 months following treatment, the true efficacy of the drug can be evaluated only by the results of examinations done through longer periods of time. If further observations prove that the disappearance of ova from stools during treatment with this drug is due to the actual death of the worms, one would then feel justified in recommending its use under careful medical supervision.





# SIMPLIFIED QUANTITATIVE METHODS FOR HOOKWORM CONTROL PROGRAMS<sup>1</sup>

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During the last twenty years the majority of surveys and other epidemiological studies of hookworm disease have been based on the use of quantitative methods, principally dilution egg counting and hemoglobin determinations. More recently these quantitative methods have been extended into a new field, namely, they are now being used to direct specific control measures towards the people most needing attention. In this extension beyond the field for which dilution egg counting was originally designed, two types of difficulties have been encountered. In the first place, the limit of accuracy of the method has been well nigh reached, or perhaps exceeded. In the second place, the statistical methods necessary for correct interpretation, while comprehensible to specialized workers, are not easily understood by nurses and other health department personnel whose training has not included an opportunity to become familiar with statistical technics.

The nature of the first difficulty can be illustrated by using the program of the Georgia Department of Public Health as an example. This program is based on the following facts and assumptions.

1. Not all persons infected with hookworm show clinical symptoms.
2. Nearly all of those showing pronounced symptoms are members of rural, white families living under the poorest economic conditions, usually as tenants or share croppers.
3. It would seem that the primary responsibility of public health agencies, as far as hookworm disease is concerned, would lie with these families rather than with the far greater number of people who are infected with relatively few hookworms and show no discernible symptoms.
4. Sanitation programs have not yet reached this type of family and certain inherent difficulties indicate that they will not be reached in the near future.
5. Alleviation of the symptoms by treatment, i.e. anthelmintics to reduce the size of the worm burden and iron compounds to increase hemoglobin production, plus an associated educational effort, are therefore being relied upon until some more permanent control measures are applicable.

It seems obvious that such a program needs a means of distinguishing which persons have symptoms resulting from hookworm infection, but no really adequate means are available. At present the procedure is to make a yearly selection of school children who show clinical signs of anemia, checking the border

<sup>1</sup> Read by title at the Fortieth Annual Meeting of the American Society of Tropical Medicine, St. Louis, Missouri, November 15, 1944.

This paper represents contribution No. 17 from the Division of Malaria and Hookworm Service, Georgia Department of Public Health, under the auspices of which these studies were made. The author wishes to thank the other members of the department for their cooperation.

line cases with hemoglobin determinations. Egg counts are then made on all anemic children. If the count of any child is above 2500 eggs per cc. (i.e. 5000 e.p.cc. when corrected to formed stool basis), his family is visited so that all members can be examined and treated if infected. This program has been successful, especially as regards keeping the children relatively free from symptoms while passing through the critical period of childhood and early adolescence, after which they seem to be less affected. Actually, what is being done in this program is to select out for attention a group of people which includes all of those who may benefit from treatment. It also includes, however, some people who show no measurable benefit from treatment, but this inclusion is inevitable until some more precise method of selection has been devised.

From this example, it can be seen that the crux of the first difficulty lies in the fact that the dilution egg count method will not in its present stage of development give an accurate estimate of the magnitude of infection of any single individual because of its high random error. Since, however, this error is usually unbiased, the method will satisfactorily determine the average egg output. Its most useful purpose in a control program is, therefore, to divide the population into groups. The limits between these groups must be set in such a way that one of these groups will contain all of the people who need immediate attention even though it also includes some people who will not necessarily benefit from that attention.

Such separation into groups has proved to be very useful but there is still need for a new method which will determine quickly and with reasonable accuracy where each individual stands on the egg output scale. Such a method would enable the health officer to determine not only which population group needs the first attention, but also in which individuals of this group the need is most urgent. Moreover, this method would also enable private physicians to more readily take a quantitative view of their cases.

As for the second difficulty, the correct analysis and summary of dilution egg count data have necessarily involved somewhat complicated statistical methods. In extending the use of the dilution egg count method to modern hookworm control programs, it is necessary to present the data to nurses and other public health personnel who have had little or no training in statistics. If the presentation is to make its point, the statistical method must be somewhat simplified. The principal purpose of the following discussion is to show how the results of the dilution egg counts can be stated in simple terms of practical value and still be sufficiently complete and precise for all statistical purposes.

#### INTERPRETATION AND USE OF EGG COUNT DATA BY THE HEALTH OFFICER

The method of presenting egg count data to be recommended can best be explained by the use of an example. In table 1 the data resulting from examination of 824 persons in Brantley County, Georgia, are presented according to this method. Since the interpretation of these data will be made in a separate paper, the present discussion will be confined to methodology. As a background for discussion, however, it may be stated that the data are based on the examination

of specimens collected from rural, white families by a visiting nurse. These families were chosen at random from over the whole county, except for the fact that obviously well-to-do families were not included. Specimens were examined by a salt flotation method and the positives were re-examined by eggcount, using the technique as modified by Stoll and Hausheer (1).

The health officer will find in the above table all of the available information which is of use to him. In column 1 the population is divided into 3 groups on the grounds of their distinct epidemiological significance, namely: pre-school children, children of school age, and adults. Any other appropriate grouping might have been used. The data of each group have been tested and found to be homogeneous. It is therefore possible to visualize with reasonable accuracy the extent of the hookworm problem in each of these three groups. The validity of conclusions can be judged from the number of persons examined, as shown in column 2. Column 3 shows the prevalence of hookworm infection in each group as indicated by the percentage of specimens found positive by flotation examination. Column 4 containing the percentage of specimens positive by egg count

TABLE 1  
*Hookworm infection in Brantley County, Georgia, in 1942*

1 AGE GROUP	2 NUMBER OF PERSONS EXAMINED	3 PER CENT INFECTED WITH HOOKWORM*	4 PER CENT POSITIVE BY EGG COUNT†	5 PER CENT OF SUS- PECTED CASES OF HOOKWORM DISEASE‡
0-4	160	49.4	45.6	23.4
5-19	514	67.7	63.8	32.6
20+	150	46.7	38.2	11.3

\* Per cent positive by flotation.

† With 200 or more eggs per cc.

‡ With 2500 or more eggs per cc.

examination is useful in making comparisons between areas. The principle reason for its inclusion, however, is that this information is absolutely essential for statistical purposes, as will be shown below. The data of column 5 are the most significant from a practical viewpoint. They are, to a certain extent, a measure of the amount of hookworm disease in a population group, and will therefore be discussed more at length.

It is now generally agreed that the pathogenicity of hookworms is fundamentally based on their blood letting activities, that anemia is the primary symptom, and that all other manifestations are secondary to it. The extent to which the severity of anemia is related to the intensity of hookworm infection is often obscured, however, by a nutritional factor. Apparently, most well nourished persons have enough immunity to prevent the establishment in the intestine of hookworms in numbers sufficient to cause appreciable anemia (2). In certain states of malnutrition this protective mechanism is weakened and large numbers of worms become established. Moreover, this nutritional imbalance may of itself cause anemia. Hookworm disease is, then, primarily the result of the addi-

tional drain which the worms place upon a hemopoietic system already nearing, if not past, its maximum limit of compensatory reaction. In evaluating the seriousness of hookworm disease, there are two factors to be considered. The first is a determination of the degree of anemia, which can be accurately done by use of a hemoglobinometer, or approximately estimated from the clinical signs. The second is a necessity for determining what portion of the anemic condition is due to the basic state of malnutrition and what portion to the superimposed hookworm infection. Hill and Andrews (3) showed that in Georgia no measurable depression of the average hemoglobin reading was associated with hookworm egg counts of less than 2500 per cc.<sup>2</sup> Above this point, however, a progressive drop in the hemoglobin reading was correlated with the size of the egg count. From these data it was concluded that in Georgia all of the cases of hookworm disease would be found in the group whose counts were above 2500 eggs per cc. In practice, the order of making these determinations is reversed (5). School children are first given a physical inspection and egg counts are then made only on those with signs of anemia. The group of anemic children with counts above 2500 per cc. should contain all of the true cases of hookworm disease as well as some who will not benefit from treatment. It is not feasible with present methods to attempt to select further, but the group to which first attention should be given has already been narrowed down to a small proportion of the population.

Returning now to our example, from the data as shown the health officer can conclude that two-thirds of the school children of these families are infected with hookworm, and that one-third of them harbor hookworms in numbers sufficient to cause any symptoms of anemia that may be noticed. Likewise, about half of the pre-school children are infected, and more than one-fifth of them have significantly heavy infections. Among adults, even though the prevalence is almost equally high, only one-tenth of the population has infections of a size to be considered of possible public health significance. Attention should be called to the fact that in this example the people were chosen for examination at random. Therefore the cases of suspected hookworm disease included in column 5 would need to be examined for signs of anemia in order to determine which ones are most in need of attention.

*Method of preparing the recommended tables:* The process of preparing table 1 is not as simple as would be thought. The chief difficulty is introduced by the fact that some specimens are not of sufficient size to make both a flotation and an egg count preparation possible. Of course, these specimens can be excluded from consideration before the flotation examination is made, but as a rule the laboratory proceeds with flotations on all specimens and then makes counts only on the positives. At this point the ones with insufficient quantity cannot be thrown out of consideration, because of the bias introduced into the data by the exclusion

<sup>2</sup> The figure actually reported by these authors was 5000 eggs per cc. F.S.B. They had used a correction factor for the consistency of the stools, reporting the data on a *formed* stool basis. The present author has determined that in Georgia the use of this factor practically doubles the average value of egg counts throughout most of the range. For purposes of simplicity and for reasons given elsewhere (4) all data in this paper are uncorrected.

of a group of positive specimens without also excluding the corresponding negative specimens. The best procedure is to obtain another specimen from the person in question and to substitute the results of its examination in the original record. This cannot always be done, however, and sometimes is too expensive to be practical. Therefore, some adjustment of the data must be made. Using the 5 $\frac{1}{2}$  to 19 age group of table 1 as an example, we first find the 514 cases to be distributed as follows:

Positive by flotation:	
With counts above 2500.....	159
With counts from 200 to 2500.....	152
Total positive by count.....	311
Negative by egg count.....	19
Total counted.....	330
Insufficient material for counting.....	18
Total positive by flotation.....	348
Negative by flotation:	166
Total examined by flotation.....	514

These figures can best be discussed in reverse order. Of the 514 specimens examined by flotation, 348 were positives, i.e. 67.7 per cent, the latter figure being entered in column 3 of table 1. Of these 348 only 330 could be re-examined by egg count and 311 were found positive, i.e. 94.2 per cent. We can assume that the 18 unexamined specimens would have been distributed in the same proportions as the ones examined, and therefore conclude that if all 348 had been examined, approximately 94.2 per cent, or 328 specimens would have been positive. Of the total 514 these 328 represent 63.8 per cent, which figure is entered in column 4 of table 1. The same result could be reached in a simpler fashion by taking 94.2 per cent of 67.7 per cent. Likewise, of the 330 examined by count, 159 were above 2500 eggs per cc., i.e. 48.2 per cent. This percentage times 67.7 per cent gives us the figure 32.6 per cent to be entered in column 5 of table 1. If the number with insufficient material is more than a small part of the total, this number should be stated in a footnote.

*Statistical interpretation of comparative data:* Although the data in the form presented above include all of the information usually needed by the health officer, they must be converted into another form for many statistical purposes. For example, in this form they cannot be directly compared with data presented in a different way, or even with data presented in the same way where different levels of significance are used. Of course, the best comparison could be made when all of the original data are available, but it is seldom possible to present data with sufficient completeness on account of limitations of publication space. Taking advantage of the fact that all homogeneous series of egg count data conform to a characteristic pattern (6), any series can be approximately reconstructed from values of the type shown in table 1. The reconstruction is based on the fact that the counts from an entire homogeneous group of positive cases approximate a normal curve when plotted on logarithmic paper. We shall continue with the same example used above to show how this normal curve can

be reconstituted from the data as presented in table 1, but first, let us consider briefly the complete series of original data of our example. For this purpose only the 330 specimens which were actually counted will be included.

Earle and Doering (7) first suggested that egg counts would approximate a normal curve if plotted on a logarithmic scale, and suggested that each series be divided into approximately 12 evenly spaced logarithmic groups. Experience with this method (8) showed that the system of gradation must be chosen with care in order accurately to space on the logarithmic scale the cases near the bottom of the range; i.e. those with only one, two, or three eggs in the quantity of material actually examined. The system of gradation which has proved to be most satisfactory is the use of a class interval of 0.2 on the logarithmic scale

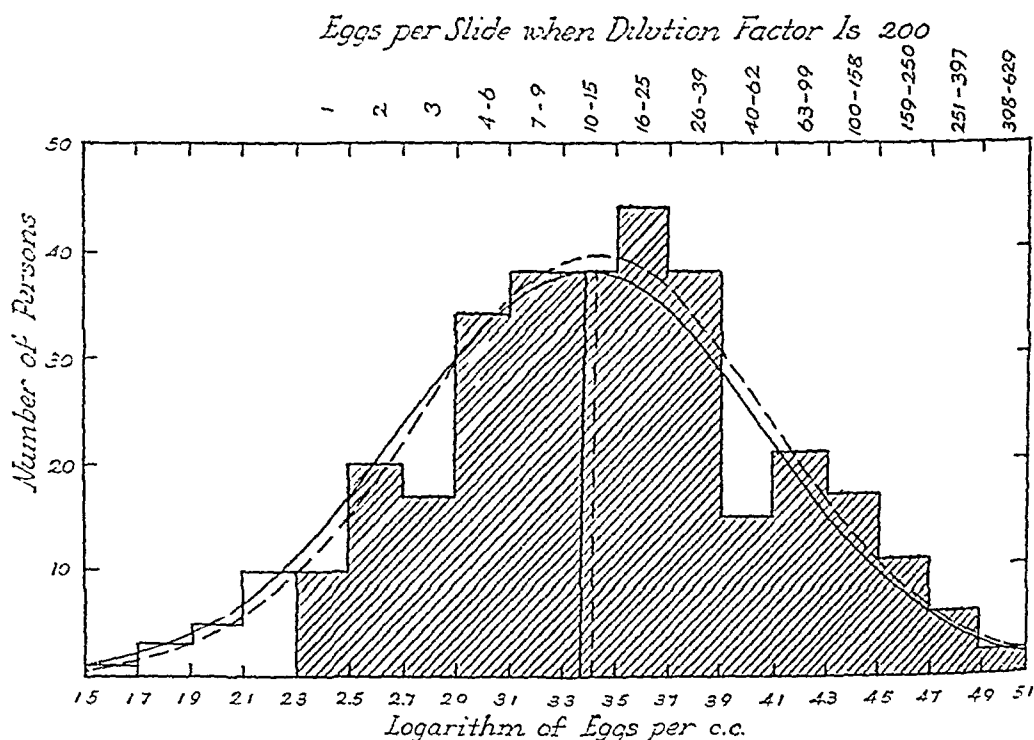


FIG. 1. SHOWING THE DISTRIBUTION OF EGG COUNTS OF POSITIVE CASES, AGE GROUP 5 TO 19 FROM TABLE 1

as shown in figure 1.<sup>3</sup> The data of the 311 cases which were positive on the egg count examination were arranged according to the groups of eggs per slide shown at the top of this figure, and were then used to plot the shaded part of the histogram. Since the 19 specimens which were negative by egg count had previously been found positive by flotation, they must be included in the normal curve.

<sup>3</sup> The only point where correspondence is not accurately approximated is in the group from 2.3 to 2.5 on the logarithmic scale. Here the value 200 eggs per cc., representing 1 egg per slide, corresponds to the lower limit of the group rather than to the mid-point, 2.4, as it should do. Since, however, this value also represents the point below which no count can fall when only one slide is counted, there is justification for considering this value to be placed at the lower limit of the group, and for using the value 2.3 as the mid-point when calculating constants, whenever an especially high degree of accuracy is desired.

Their distribution was determined by extrapolation from a line fitted to the data plotted on probability paper and is shown as the clear part of the histogram.

The normal curve shown by dotted lines was then fitted to the entire group of data by least squares and provides us a basis for the comparison of other approximations. The curve shown as a solid line is an approximation read from a straight line fitted on probability paper to two points determined by the values of columns 4 and 5 of table 1.<sup>4</sup> When the two normal curves are compared it is obvious that the approximation from two points does not fit the data quite as well as does the curve fitted from the entire series, but the difference between the two is insignificant in view of the fact that the original data only roughly approximate a normal curve. As a matter of fact this particular example happens to be considerably more variable than the average series of egg counts. In any case what is desired is merely an approximate curve from which values can be read at any point so as to make possible a comparison with other series of data.

#### SUMMARY

Hookworm egg count data from south Georgia are used to illustrate a simple method of presenting such data in a form easily interpreted, yet complete and precise. The data are listed in the following categories for each population group: number of persons examined, percentage positive by flotation examination, percentage positive by egg count examination, percentage with counts above some specified level.

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<sup>4</sup> A slight confusion is introduced by the 19 cases which could not be examined by egg count. In practice one would reconstruct the curve on the basis of the entire 514 cases of which 67.7 per cent, or 348 cases, were considered to be positive by egg count, and 32.6 per cent of which, or 168 cases, were considered to be above 2500 eggs per cc. These 328 and 168 cases represent respectively 94.3 and 48.3 per cent of 348, which points would be plotted at the 2.3 and the 3.4 levels on the logarithmic scale of probability paper for determining the straight line. Since for purposes of comparison we must use only the 330 cases actually counted, the values 94.2 and 48.2 previously calculated were used here. The difference is not significant in light of the variability involved.





## TREATMENT OF HYMENOLEPIS NANA INFECTION WITH "ACRANIL"\*

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In a recent communication (1945) we reported on the treatment of 50 cases of *Giardia lamblia* infection with "Acranil" which proved to be a highly effective chemotherapeutic agent for the treatment of this infection. That study was carried out during 1938-1939 on cases discovered among 300 children in two orphanages. Pertinent data on the incidence of other protozoal and helminthic infections of these children were given in the paper referred to; to avoid repetition they will not be dealt with again. During the same survey 25 of 27 cases of *Hymenolepis nana* infections were also treated with "Acranil." Acranil is an acridine derivative, closely related to "Atabrin" or "Quinacrine." The 25 cases of *Hymenolepis nana* infections were treated as follows:

The 25 children were divided into four groups, according to their ages: On the evening before beginning the course of treatment with Acranil, 0.1-0.2 gm. calomel was administered to each child as an aperient. The following morning Acranil was given on empty stomach in a single dose. Tablets were swallowed with some water. Three hours later a sodium sulfate purge was administered. Food was served after the first or second bowel movement. Treatment with Acranil was continued in smaller doses for three more days without any further purgation. On the whole, Acranil was very well tolerated by the children. Four children vomited the tablets soon after swallowing, but upon repeating the dose the tablets were retained. None showed any untoward effects.

*Group I.* 10 children, between the ages 4-8, were given each two 0.1 gm. tablets of Acranil, early in the morning, on empty stomach. Three hours later a sodium sulfate purge was given. Then, for three consecutive days, each child swallowed one 0.1 gm. tablet of Acranil after breakfast.

*Group II.* 9 children, between the ages of 8-10, were each given three 0.1 gm. tablets of Acranil, early in the morning, on empty stomach. Three hours later a sodium sulfate purge was given. Then, for three consecutive days, each child swallowed one 0.1 gm. tablet of Acranil in the morning and another one in the evening after each meal.

*Group III.* 4 children, between the ages 11-14, were each given four 0.1 gm. tablets of Acranil, early in the morning, on empty stomach. Three hours later a sodium sulfate purge was given. Then, for three consecutive days, each child swallowed one 0.1 gm. tablet of Acranil three times daily after meals.

*Group IV.* 2 girls, 14 and 16 years old respectively, were each given five 0.1

\* Acranil was provided by the manufacturer, the "Bayer" firm, Leverkusen, for these experiments.

gm. tablets of Acranil, early in the morning, on empty stomach. Three hours later a sodium sulfate purge was given. Then, for 3 consecutive days, each girl received one 0.1 gm. tablet of Acranil after each meal.

Weekly stool examinations were made on these children for five consecutive weeks after the administration of the treatment. Five months after treatment a final sixth stool specimen was also examined. Stools were examined by "Direct Smear," and by the "hydrochloric acid and ether concentration" method of Theleman.

It is evident from the data presented in table 1 that Acranil is very effective against the adult *H. nana*. One week after the treatment only one out of 25 children passed ova of *H. nana* in his stools. Two weeks after the treatment 2

TABLE 1  
*Treatment of Hymenolepis nana infections with Acranil*

AGE	NO.	TREATMENT WITH ACRANIL	POSITIVE STOOL EXAMINATIONS					
			7 d.	14 d.	21 d.	28 d.	35 d.	5 mos.
4-8	10	0.2 gm. at once on 1st day; then 0.1 gm. once a day for 3 days.	0	0	0	1	1	4
8-10	9	0.3 gm. at once on 1st day; then 0.1 gm. twice daily for 3 days.	0	1	2	5	6	4
11-14	4	0.4 gm. at once on 1st day; then 0.1 gm. thrice daily for 3 days.	0	0	0	0	1	3
14-16	2	0.5 gm. at once on 1st day; then 0.1 gm. thrice for 3 days.	1	1	1	1	2	1
Total...	25 cases		1 = 4%	2 = 8%	3 = 12%	7 = 28%	10 = 40%	12 = 48%

children passed ova. Three weeks after the treatment 3 children passed ova in their stools. In other words, three weeks after the administration of the treatment, about 88% of the cases treated were negative. After the third week the number of positive stools sharply increased, so that at the end of the fourth week after treatment, seven children showed ova in their stools and five months later 12 out of 25 children were found to be reinfected.

#### DISCUSSION

*Hymenolepis nana* is the most common tapeworm of man in Southern Europe and U. S. A. Sunkes and Sellers (1937) collected data on 927,625 fecal examinations in the Southern United States, performed during 1931-35, and found that

out of 8085 tapeworm infections, 7,149 were *H. nana* infections. This report included 11 surveys from states in which the incidence of *H. nana* infection varied from 0.39-2.7%. In some parts of India Chandler (1940) found the incidence to be as high as 18-28%. Yenikomshian and Berberian (1934) reported that in Syria and the Lebanon the incidence was 1.5 per cent. In the orphanages where this study was carried out the incidence of *H. nana* infection was 9%.

*Hymenolepis nana* is the only cestode of man which does not need an intermediate host. The eggs that may be ingested in food or drink hatch in the duodenum or jejunum. The embryonic oncospheres actively penetrate the villi and there they develop into larvae known as *cysticercoids* or *cercocystis*. When mature, the larvae break away from the villi and establish themselves lower down in the lumen of the ileum near the ileocecal valve and grow into adult worms. The period from the date of the ingestion of eggs to the time of first appearance of ova in the stools is known as the *prepatent period*; and the time from the first appearance of the ova in the stools and the spontaneous loss of worms is known as the *patent period*. Grassi (1887) believed that the dwarf tapeworms are lost in 35 days after infection. Joyeux (1920) and Woodland (1924) stated that this time interval is 30 days or less. The life history of *Hymenolepis fraterna* of the rat was thoroughly studied by Shorb (1933) and Hunninen (1935). The murine form of the dwarf tapeworm is identical with the human form in structure and life history. Experiments have shown that the rodent form is infectious for man and the human form for rats and mice. Although according to some workers the incidence of cross infections is low, many authors accept the identity of these two species. Shorb (1933) found that the ova of *H. fraterna* are most viable and infective when freshly passed. The viability is gradually lost. When ova are stored in water, they are no longer viable after 11 days. Hunninen (1935) found that 35% or more of ingested eggs are passed out unchanged in the feces, a large percentage of which are still viable. The percentage development of cysticercoids is on the average 4.1 and of adults 2.8%. The cysticercoids leave the villi at approximately 102 hours and at the end of 144 hours almost all have escaped. According to Hunninen the prepatent period may vary from 14-25 days, and he considers it to be usually 15 days. The patent period is variable. In the rat and mouse this appears to be short, less than 11 days (Shorb).

Concerning the treatment of this important tapeworm of man there is only a casual reference in standard text books on tropical medicine and parasitology. Filix mass, oil of chenopodium, hexyl resorcinol, carbon tetrachloride combined with oil of chenopodium are mentioned as being more or less effective against dwarf-tapeworm. In Manson-Bahr's experience (1942) it has not been easy to dislodge this tapeworm with any of these anthelmintics. On account of their toxicity, filix mass, carbon tetrachloride and oil of chenopodium cannot be used repeatedly with safety, hence they cannot be considered suitable for the treatment of *H. nana* infection. Kutschinsky (1933) treated five cases of dwarf tapeworm infection in mice with osarsol (stovarsol) by giving two daily doses for 3 days and claimed the disappearance of eggs for 4 months and longer. In the experience of Maplestone and Mukerji (1939) stovarsol failed to cure *H. nana*

infection in man; they treated 12 cases with gentian violet with promising results. Their observations indicated that gentian violet given in 1 grain t.i.d. doses for a week, or several courses of three days' duration at weekly intervals, was the only treatment of real value. Culbertson (1940) reported that 0.01 gm. of atabrine given by mouth on 2 successive days and somewhat smaller doses given for prolonged periods, generally completely cured *Hymenolepis fraterna* infection in mice. He stated that subcurative doses of the drug, too small to cause the elimination of all the parasites suppressed for a time thereafter the egg production by the worms, but this function was resumed as the effect of the drug wore off.

If we analyse our results in the light of the above facts, we may be justified in drawing the following conclusions:

1. That the treatment with Acranil eliminated the egg laying mature dwarf tapeworms in 24 out of 25 cases of infection as revealed by stool examinations performed one week after the treatment.

2. Though the effectiveness of Acranil on the cysticercoids of *H. nana* could not be gauged directly from stool examinations, it would appear that the drug must have been equally effective against them, as 14 days after treatment only two out of twenty five cases treated passed ova in their stools and the rest remained negative (92%). The prepatent period of *H. nana* being 15 days, the cysticercoids which were not eliminated by the treatment would have had ample time to develop into maturity and their ova would have appeared in the stools. After 15 days it is no longer possible to eliminate the possibility of reinfection.

It is therefore reasonable to conclude that of all the anthelmintics hitherto used against the dwarf tapeworm of man, Acranil is perhaps the most effective, the least toxic and one that can be administered with ease.

#### SUMMARY

25 cases of *Hymenolepis nana* infection in children were treated by a synthetic acridine derivative "Acranil." The night before the administration of Acranil, the children were each given 0.1-0.2 gm. Calomel as aperient. The following morning Acranil was given on an empty stomach in 0.1-0.5 gram doses according to age. Three hours later a saline purge was administered. Treatment with Acranil was continued in smaller doses for 3 more days without any further purgation. 23 out of 25 children treated by this method remained free of worms for 14 days.

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# EMERGENCY STERILIZATION OF DRINKING WATER WITH HETEROPOLAR CATIONIC ANTISEPTICS

## I. EFFECTIVENESS AGAINST CYSTS OF *ENDAMOEBA HISTOLYTICA*<sup>1,2</sup>

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Although there are many practical uses for antiseptics which are effective against cysts of *Endamoeba histolytica*, one of the most important and difficult is the emergency sterilization of drinking water under military combat conditions. Various halogen preparations have not been entirely satisfactory because they tend to be inactivated by organic nitrogenous material and by alkalinity. Since natural waters encountered in the field may sometimes contain very high concentrations of organic nitrogenous material or may be alkaline, and since military emergency conditions in the field require uniform dosage, it is necessary to use the halogens in high initial concentrations under all conditions so that they may prove effective even under the worst conditions. These high concentrations of the halogens are distasteful and may not be entirely without toxicity.

The antiseptic activity of certain heteropolar cationic compounds, or synthetic, cationic detergents, was described in 1935 by Domagk (1) and Katz and Lipsitz (2). These compounds are surface-active agents in which the active portion of the molecule is the cation. A low toxicity of certain of these chemicals had been reported in animals (1,3-10), numerous reports had appeared indicating their effectiveness against various bacteria, and Roccal had been found to be effective against cysts of *Endamoeba histolytica* (11).

Preliminary experiments, conducted in this laboratory during 1943, indicated that three chemicals of this type, Zephiran, Phemerol, and Ceepryn, were able to kill amebic cysts in dilutions of at least 1:10,000 with a cyst density ranging up to 10,000 cysts per ml. of solution and with a time of exposure of 10 minutes at 20°C. Similar preliminary experiments with bacteria indicated that the drugs remained apparently germicidal over a wide pH range and that they were not markedly affected by concentrations of organic nitrogen of more than 1,000 parts per million. Concentrations of some of these chemicals which were found effective against amebic cysts and vegetative bacteria did not seem unduly distasteful. Many heteropolar cationic antiseptics were said to be stable and obtainable in a solid but freely soluble form. It therefore seemed possible that chemicals of this general type might be more useful than the halogens for the emergency sterilization of drinking water, as well as for other cysticidal purposes.

<sup>1</sup> The work reported in this paper was conducted under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Southern California.

<sup>2</sup> Read at the Annual Meeting of the American Society of Tropical Medicine at Cincinnati, Ohio, November 14, 1945.

A survey (12) of a large number of compounds of this general chemical type has been carried out to determine their effectiveness against *Escherichia coli*, *Staphylococcus aureus*, aerobic bacterial spores, and several strains of fungi and to estimate their acute toxicity when administered to mice orally or intraperitoneally. That survey indicated that heteropolar cationic compounds might prove to be excellent antiseptics. In the present communication are shown the results obtained with 48 of these chemicals against cysts of *E. histolytica*.

#### MATERIALS AND METHODS

Suspensions of cysts of the NRS strain of *E. histolytica* were prepared as previously indicated (13). Direct microscopic counts were made of these suspensions which were then adjusted to contain approximately 10,000 cysts per ml. Stock suspensions of cysts were kept at 4°C. and used between the first and tenth days of preparation.

Multiple geometric dilutions, in distilled water, of the antiseptic to be tested were prepared in volumes of 9 ml. in cork-stoppered centrifuge tubes, brought to 20°C. in a water bath, and inoculated with 1 ml. of the standard cysts suspension. After about 8 minutes the tubes were centrifuged, and at the end of 10 minutes, the supernatant fluid was siphoned off and sterile water added. After mixing, the solution was again centrifuged, and this washing process was carried out a second time before the entire sediment was inoculated into RES medium containing starch and the bacteria which were present in the original amebic culture. Each culture tube was examined repeatedly over a period of 5 days for the appearance of trophozoites. If none were found, it was concluded that the cysts had been killed during contact with the antiseptic.

Preliminary trials were run in duplicate with 0.3 to 0.5 log differences in concentration. Further titrations were conducted in triplicate with 0.1 or 0.2 log differences in concentration when the  $ED_{50}$  of the preliminary test appeared to be 1:10,000 or more. Antiseptics having end-points of 1,20,000 or more were tested in quintuplicate at least and with 0.1 log changes in concentration from one dilution to the next.  $ED_{50}$  end-points,<sup>3</sup> or dilutions which were apparently cysticidal in half the trials, were calculated according to the methods of Reed and Meunch (14).

#### RESULTS

Table 1 indicates the chemical name and the dilution ( $ED_{50}$ ) found effective in half of the trials against approximately 1,000 cysts of *E. histolytica* per ml. in an exposure period of 10 minutes at 20°C.

It is seen that under the conditions of test the majority of these compounds were effective in dilutions of at least 1:5,000; that 20 of them were effective in dilutions of at least 1:20,000; and that 3 of them were effective in dilutions of over 1:80,000.

<sup>3</sup> In calculating these end-points, dilutions before inoculation, rather than final dilutions after inoculation, have been used. The final dilution was 11% higher than the starting dilution because 1 ml. of inoculum was added to 9 ml. of the antiseptic.



TABLE 1

OUR NO.	MANUF. <sup>a</sup>	FORMULA	CYST ED <sub>50</sub>
35	C	n-hexadecyl trimethyl ammonium bromide ("Cetamium")	70,800
48	O	n-hexadecyl dimethyl 2-hydroxyethyl ammonium chloride	87,100
49	O	n-hexadecyl diethyl 2-hydroxyethyl ammonium chloride	33,900
51	O	n-hexadecyl di-n-propyl 2-hydroxyethyl ammonium chloride	20,000
50	O	n-hexadecyl di-n-butyl 2-hydroxyethyl ammonium chloride	85,100
4	W	dimethyl 9-octadecenyl ethyl ammonium bromide	10,000
5	W	dimethyl 9-octadecenyl ethyl ammonium bromide (technical crude)	14,100
1	W	alkyl dimethyl benzyl ammonium chloride ("Zephiran") (C <sub>8</sub> to <sub>18</sub> )	17,800
10	W	Industrial grade "Zephiran" ("Roccal")	20,000
2	W	Technical crude "Zephiran"	40,700
3	W	alkyl dimethyl benzyl ammonium chloride (C <sub>11</sub> to <sub>18</sub> )	41,700
20	U	di-n-octyl methyl benzyl ammonium bromide	10,200
44	U	dioctyl methyl cinnamyl ammonium chloride	19,500
30	PD	(p-octyl phenoxy ethoxy ethyl) trimethyl ammonium chloride	5,000
9	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl benzyl ammonium chloride ("Phemerol")	17,800
25	PD	(p-butyl o-bromo phenoxy ethoxy ethyl) dimethyl benzyl ammonium chloride	<5,000
27	PD	(p-cyclohexyl phenoxy ethoxy ethyl) dimethyl benzyl ammonium chloride	5,000
29	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl p-chlorobenzyl ammonium chloride	46,800
28	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl cinnamyl ammonium chloride	>20,000
32	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl benzyl ammonium butyrate	14,100
33	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl benzyl ammonium octyloxyacetate	14,100
31	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl benzyl ammonium undecylate	38,000
34	PD	(p-octyl phenoxy ethoxy ethyl) dimethyl benzyl ammonium oleate	10,000
14	U	1-n-tetradecyl pyridinium chloride	20,900
39	U	1-n-tetradecyl 3-methyl pyridinium bromide	7,080
16	U	1-n-tetradecyl 3-carbamyl pyridinium bromide	56,200
15	U	1-n-tetradecyl 3-diethylcarbamyl pyridinium bromide	8,910
13	U	1-n-tetradecyl 4-methyl pyridinium chloride	182,000
18	U	1-n-tetradecyl 4-diethylcarbamyl pyridinium bromide	7,080
11	M	1-n-hexadecyl pyridinium chloride ("Ceepryn")	74,000
12	U	1-n-hexadecyl 2-methyl pyridinium bromide	19,950
41	U	1-n-hexadecyl 3-methyl pyridinium bromide	14,100
17	U	1-n-hexadecyl 3-carbamyl pyridinium bromide	21,600
40	U	1-n-hexadecyl 3-diethylcarbamyl pyridinium chloride	7,080
52	E	1-(n-undecyl formoxy ethyl carbamyl methyl) pyridinium chloride ("Emulsol")	5,620
53	E	1-(n-tridecyl formoxy ethyl carbamyl methyl) pyridinium chloride ("Emulsol")	8,510

TABLE 1—*Concluded*

OUR NO.	MANUF.*	FORMULA	CYST. ED <sub>50</sub>
54	E	1-(n-pentadecyl formoxy ethyl carbamyl methyl) pyridinium chloride ("Emulsol")	8,710
55	E	1-(n-heptadecyl formoxy ethyl carbamyl methyl) pyridinium chloride ("Emulsol")	8,320
22	U	1-benzyl 4-n-undecyl pyridinium chloride	7,080
42	U	1-allyl 4-n-tridecyl pyridinium bromide	28,800
45	U	1-benzyl 4-n-pentadecyl pyridinium chloride	4,070
43	U	1-benzyl 4-n-dodecyl-carbamyl pyridinium chloride	2,190
47	O	1-n-hexadecyl 1-(2-hydroxyethyl) piperidinium chloride	37,200
46	O	1-n-hexadecyl 1-(2-hydroxyethyl) morpholinium chloride	58,900
21	U	1,3-di-n-heptyl benzotriazolium bromide	<5,000
26	PD	1,3-di-n-octyl benzotriazolium bromide	>42,700
38	U	1-n-dodecyl 3-ethyl benzotriazolium bromide	<5,000
23	U	1-n-tetradecyl 3-ethyl benzotriazolium bromide	10,000

\* C: Cetamium; O: Ortho; W: Winthrop; U: Upjohn; PD: Parke, Davis; M: Merrell; E: Emulsol.

Comparison of effectiveness of these antiseptics against amebic cysts, as shown above, and *Escherichia coli*, as noted elsewhere (12), indicates that they generally tended to be somewhat more effective against *E. coli* than against amebic cysts. This relationship was by no means constant, however. In the case of 38 compounds in which it was felt that both end-points had been determined with sufficient precision for purposes of such a comparison, the ratio of effectiveness against *E. coli* compared with effectiveness against cysts (coli ED<sub>50</sub> divided by cyst ED<sub>50</sub>) was found to have a mean value of 1.7, a mode of 1.5, and a median of 1.4. One chemical was found to be over 7 times as effective against amebic cysts as against *E. coli*, while at the other extreme 2 compounds were found to be more than 5 times as effective against *E. coli* as against cysts. In the case of half of the 38 compounds, the effectiveness against cysts was less than twice and more than half the effectiveness against *E. coli*. It therefore seems possible, on the basis of these data, to state that the "cysticidal" power of an antiseptic of this type may differ within considerable limits from its "bactericidal" power.

The acute oral toxicity of these antiseptics for mice has also been indicated (12). From those data, in comparison with the results noted above, it is seen that many of these compounds provided a moderate or considerable margin of apparent safety. The safest compound studied was n-hexadecyl dimethyl 2-hydroxyethyl ammonium chloride (Ortho #244, our #48), in which the ED<sub>50</sub> for cysts was 1:87,100, and a dilution of 1:50 administered orally in a single dose of 0.5 cc. to white mice failed to kill half of the animals within a period of 10 days. This particular compound, however, was not particularly effective against *E. coli*, the ED<sub>50</sub> for that organism being 1:20,400.

A few of the more effective and less toxic compounds were sampled for taste, and it was found that they had a slightly medicinal, slightly astringent taste in concentrations which were effective against both *E. coli* and amebic cysts.

## DISCUSSION

The heteropolar cationic antiseptics, sometimes called synthetic cationic detergents, have been found effective against cysts of *E. histolytica* in vitro. These results are in agreement with Wright's observations (11) and are consistent with the confirmatory results recently published by Fair (15). There are two principal problems involved in their use for the sterilization of drinking water, however. They have a definite and slightly unpleasant taste in effective concentrations, and these concentrations have not been examined sufficiently to demonstrate their complete lack of toxicity when consumed over prolonged periods of time by man. Further studies were therefore undertaken (16) in an effort to find some method for the inactivation of excess antiseptic after its antibiotic action had taken place.

The cysticidal activity of heteropolar cationic antiseptics suggests consideration of their use also for other practical purposes, such as decontamination of dishes, fruits, vegetables, clothing, body surfaces, and the like.

## CONCLUSIONS

1. A large number of heteropolar cationic antiseptics have been found sufficiently effective against bacteria and cysts of *E. histolytica* in vitro to justify considering their use for the emergency sterilization of drinking water in canteens and for other cysticidal and antiseptic purposes.

2. A minimal taste and a theoretical risk of toxicity make it advisable to remove excess chemical from drinking water after antiseptic action has occurred.

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# PRECAUTIONS BY THE ARMY TO PREVENT THE INTRODUCTION OF TROPICAL DISEASES\*

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Since the start of the war it has been universally recognized that troops deployed in the tropical theaters of operation would be extensively exposed to a variety of diseases which either do not occur in the United States or are not widely prevalent. Much speculation has taken place as to the disease problems which might arise when the Army was demobilized and troops brought home and returned to civil life. From time to time representatives of the Army, Navy, and U. S. Public Health Service have consulted to determine precautions which should be enforced to prevent the introduction of exotic diseases. Since the time for demobilization of the forces which campaigned in the Pacific, Africa, the Middle East and the Orient has now arrived, it is appropriate to evaluate the extent to which various tropical diseases have been a problem among troops and to review the measures which have been adopted by the Army to prevent introduction of these diseases by men returning from overseas.

First of all, it should be pointed out that high standards of preventive medicine have been enforced among troops serving overseas. In general, good sanitation has prevailed. Also, troops serving in endemic areas have been immunized against certain of the tropical infections; such as yellow fever, cholera and plague. These and other factors have prevented many of the diseases which afflict native populations from attaining significant incidence among military forces. This is true, for example, of such diseases as yaws, leprosy, trypanosomiasis, leishmaniasis, onchocerciasis, as well as many others which consequently will not constitute a significant hazard in returning troops. The tropical infections which have been the most important to the Army as a whole are malaria, dysentery, and various dermatoses; dengue, sandfly fever, scrub typhus, filariasis, schistosomiasis, and hookworm have at times constituted disease problems of military significance among smaller groups of men and in more limited areas. The importance of these conditions in relation to the return of troops will be discussed briefly. Since the dermatoses which have been most troublesome are related principally to the tropical environment, this problem will not be a lasting one after men are brought back to this country.

Malaria has been by far the most important disease problem among troops which served in warm climates, and the possible establishment of new foci and new strains of parasites in the United States has attracted considerable attention. Loose estimates running up into the millions have been made as to the number of

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men who might return to this country infected with malaria. Statistics may now be quoted which show that actually there were a total of 430,000 hospital admissions for malaria in the Army during the period December 1941 through June 1945. Of these, 362,000 occurred abroad and 68,000 in the United States, nearly all of the latter representing relapses of infections acquired overseas. Since these figures include readmissions, the actual number of individual infections must be considerably smaller. (Admissions in the Philippine Islands before the reoccupation in October 1944 are not included because of the absence of records.)

The Army has taken definite steps to minimize the hazard of secondary cases of malaria arising from soldiers who may still harbor malarial parasites after their return to the United States. Adequate control of anopheline mosquitoes is maintained at military installations, including hospitals, throughout the country. In the past year the program of the U. S. Public Health Service for malaria control in war areas has been extended to include selected locations in southern states where the risk of transmission is considered greatest. Evidence of effectiveness of this coordinated mosquito control program is afforded by the extremely low malaria rate which has prevailed among troops in this country and also by the fact that up to the present, as far as is known, only an insignificant number of secondary cases of malaria have been traced to oversea veterans.

Although soldiers who have had malaria will receive adequate treatment before discharge, it is inevitable that many may still harbor latent infection and may suffer a relapse at a later date. Many of the troops serving in highly malarious regions have been given suppressive medication with atabrine. Such treatment does not prevent infection even though symptoms are suppressed while the drug is taken. When the drug is discontinued delayed primary attacks occur in the majority of those who have become infected with vivax malaria, usually within a month or two after medication is stopped, although occasionally the interval may be prolonged to a year or more. Infections with falciparum malaria are usually cured provided suppressive treatment with atabrine is continued for four weeks after the last exposure.

In order to prevent attacks of malaria while men are on furlough or are traveling to new locations, it is required that soldiers who are still taking suppressive medication when they return to the United States will continue to take the drug for an additional period of 28 days. This measure is not only beneficial to the individual in preventing relapses at an inconvenient time, but also eliminates the chance of spread to others during the period of travel and insures the taking of sufficient drug to cure falciparum malaria in case infection has occurred immediately prior to return to this country.

At the time of separation from service the following procedures are carried out during the terminal physical examination to minimize the public health hazard from carriers of malarial parasites:

1. Individuals who, during the previous two years, have had malaria or have served in a hyperendemic area and have taken suppressive treatment are warned that they may have a relapse or an initial clinical attack. They are instructed

to seek prompt medical attention and to have a blood examination for malarial parasites in case of a febrile illness.

2. When an individual has had repeated attacks of malaria, he is examined for splenomegaly and other signs of chronic malaria.

3. When an individual has had an attack of malaria within the previous three months or has discontinued the taking of suppressive treatment within the previous thirty days or when symptoms or signs suggest the presence of acute or subclinical malaria, a thick blood film is examined for parasites. All individuals found positive are treated until free of symptoms and until two negative thick blood films have been obtained at two-day intervals.

Amebiasis is another disease which has occurred widely in the Army overseas, especially in India, China and the Philippines, and which may present a problem after troops have returned. Undoubtedly many infections will not have been recognized and treated. Others that have been treated may later recur. Since it is generally agreed that from 5 to 10 per cent of the population in this country is already infected with *Endamoeba histolytica*, it does not appear that the return of carriers among soldiers will greatly affect the public health problem which now exists unless strains from abroad should prove more virulent than those already present. No evidence of this has been demonstrated.

It is not considered feasible that the Army endeavor to discover and treat every individual infected with *E. histolytica* before discharge. The laboratory and hospital facilities required, as well as the delay in the process of separation, make such a procedure out of the question. An attempt is being made, however, to insure that individuals who have had amebic dysentery have received adequate treatment before discharge. When an individual gives a history of a clinical attack within the previous six months which is suspected of being due to *Endamoeba histolytica* or when symptoms or signs suggest the presence of active amebic dysentery, a freshly passed stool specimen is examined for *E. histolytica*. Individuals found positive either for trophozoites or for cysts are treated.

In the case of bacillary dysentery, stool cultures by the rectal swab technique are made when a history is given of an attack within the previous month which is suspected of being due to bacillary dysentery. Surveys have shown that the incidence of carriers of pathogenic bacteria is extremely low in returned military personnel. Apparently, use of sulfonamides for treatment has markedly reduced the persistence of *Shigella* organisms following acute attacks.

Since dengue, sandfly fever, and scrub typhus are limited in their course and do not have recurrences, the chance of their importation by returning service personnel is small. The hazard from dengue is the greatest. Outbreaks of the disease have occurred from time to time among troops at Pacific stations which are within one or two days' flying time of the continental United States. Although quarantine procedures are an effective barrier against the entry of persons sick with dengue, it is possible that an individual might arrive during the incubation period and enter undetected. Hence, control of *Aedes aegypti* is an integral part of the antimosquito program at Army bases and airports.

Hookworm infection acquired by troops serving in the Pacific and in Asia is a matter of importance to public health in the United States because of the possibility of introducing *Ancylostoma duodenale*, a species which heretofore has not been established in the southern states. Reports have indicated that *A. duodenale* frequently is the predominating species in infections acquired in the Solomons, New Guinea, the Philippines and in Burma where some combat units have shown up to 25 per cent incidence of hookworm infection. The infections, however, rarely are severe enough to cause clinical manifestations. *Ancylostoma* presents a greater public health problem than *Necator americanus*, the species now established in the southern United States, because it is more harmful to the host, is less amenable to treatment, and because its free-living stages are more resistant to climatic conditions.

An attempt to remove every single hookworm from troops returning to the United States is not considered practicable because of the difficulties of examining and treating such a large group of men and especially because repeated treatment may be necessary to effect complete cure. However, the possibility of *A. duodenale* becoming established in extensive areas in this country will be materially lessened if the number of worms introduced by returning troops is kept to a minimum. Consequently, survey and treatment of troops, especially those in combat units most exposed to infection, is encouraged before departure from abroad. Also, at the time of discharge individuals who have been treated for hookworm within the previous six months are examined and given additional treatment if hookworm eggs are found.

Other helminth parasites are not of particular concern from a public health point of view either because they are already common in this country, for example, *Ascaris lumbricoides* and *Trichuris trichiura*, or because there is apparently no possibility of their spread. More than a thousand cases of schistosomiasis japonica occurred in troops during the invasion of Leyte in the Philippines. These individuals are being given thorough treatment and follow-up examinations. In any event, since suitable snail vectors are not known to occur in the United States, it is not considered that the return of such cases presents a public health hazard.

Although several thousand infections with filariasis have occurred in Army personnel in the Pacific, only a small number of those infected has ever shown microfilariae in the blood. It has been generally agreed by representatives of the Army, Navy and U. S. Public Health Service that the chance of transmission of filariasis in the United States is very slight and that no restriction of the location or movement of individual carriers of microfilariae is necessary. The Army is maintaining a follow-up of men infected with filariasis to determine whether any appreciable number will show microfilariae in the blood at a later date.

In addition to the special precautions discussed above in relation to individual diseases, it should be pointed out that general quarantine measures have been improved and strengthened during the war. A joint Army, Navy, and Public Health Service Quarantine Board has established a program of military quaran-



tine which takes maximal advantage of the broad policy of immunization required in the armed services and the continuous medical supervision exercised over military personnel. Troops are examined before departure from abroad and after arrival in this country to identify and screen out for treatment individuals who may have acquired communicable diseases during oversea service. Also, new regulations have been formulated which are intended to prevent the introduction of disease through infected animals, plants or insects. Every feasible effort is being made to safeguard this country from new disease problems which might result from the world-wide deployment of the armed forces.



## BOOK REVIEWS

*Pathology of Tropical Diseases, an Atlas*, by J. E. Ash, Colonel, MC, USA, Director, Army Institute of Pathology, Army Medical Museum, and Sophie Spitz, MD, CS, AUS, Pathologist, Army Institute of Pathology, Army Medical Museum. 941 illustrations, 15 in color, on 257 plates. W. B. Saunders Company, Philadelphia and London, 1945.

Students of tropical medicine in many parts of the world will greet this important work with enthusiasm and will read it with intense satisfaction. The resources of the Army Medical Museum have provided unique material for photographic reproductions which the authors have elucidated by legends and annotations summarizing current scientific opinion as well as their own valuable observations. The text is rightly subordinated to the plates, which are there in abundance, picturing just the things one wants to see and has difficulty finding elsewhere, the microscopic diagnosis of stool smears (pp. 106-108) the tissue changes of histoplasmosis (pp. 184-185) or the differentiation of intracellular organisms (p. 205). The material, the photography, the photo-engraving and the presswork excite admiration. They are the best in the field of tropical pathology since the appearance of Thompson and Robertson's *Protozoology* in 1929.

If urged for suggestions for the improvement of a second edition, one recommendation would be made, that the authors consider the insertion of transparent overlays with diagrams, to increase the teaching value of the more important plates. Unretouched photographs are necessary to show what one actually finds in the microscope, but a diagram gives the inexperienced man the trained pathologist's interpretation of what he is looking at. A few textual and orthographic errors need correction. Four years is not the limit of the period of infectivity of ticks carrying relapsing fever organisms (p. 69). Untreated hookworm infection is not persistent "for years", in the absence of reinfection (p. 229). The history of arctic and antarctic exploration does not support the view that scurvy was practically eliminated by the empiric use of lime juice long before vitamins were known (p. 314). *Haemogogus capricornii*, *Rhipicephalus sanguineus*, *Rickettsia rickettsi*, *Iodamoeba bütschlii* and *Xenopsylla cheopis* are misspelled in the text (pp. 1, 33, 79 and 114). These few errors of detail do not lessen the high scholarly and scientific value of this unique and noteworthy contribution to pathology and to tropical medicine, a credit to the authors, to the Army Medical Department and to American science as a whole.

ELLISTON FARRELL

*Protozoology*, by RICHARD R. KUDO, D.Sc., Professor of Zoology, the University of Illinois, Urbana, Illinois. Seven hundred seventy eight pages with 336 illustrations. Third edition, Charles C. Thomas, Springfield, Illinois, 1946.

This edition of Kudo's text, like its prototype, the *Handbook of Protozoology* published in 1931, presents introductory information on the common free-living and parasitic protozoa for the use of zoology students of college and university grade. Part I, comprising 186 pages, is devoted to the general biology of protozoa. Taxonomy and special biology occupy Part II, which fills two thirds of the book, the collection, cultivation and observation of protozoa being considered in the final chapter. An author and subject index concludes the volume. Of the illustrations, 4 are in color, the remainder in black and white.

The book will interest teachers and students of general protozoology, for whom the book has been written. It is not intended to be an adequate guide to clinical protozoology. Some of the information about human parasitic protozoa is obsolete or deficient. The outmoded term "haemozoin" for instance is applied to malarial pigment now identified as hematin. The statement appears that "A. gambiae . . . now seems to be under control (in Brazil)"; actually the species was eradicated from Brazil in 1940. Therapeutic malaria is described as "a late utilization of the malarial organism in combatting another disease" although von Jauregg's speculations on malaria therapy date from 1887 and his clinical trials from 1917. The 1944 revision of Craig's monograph on amebiasis is apparently unknown to

the author who quotes from the earlier edition of 1934. No reference is made to the use of Field's stain in the diagnosis of malaria or to the zinc sulphate technic of stool examination for protozoan cysts.

The only serious errors noted were the statements that vivax paroxysms recur every third and quartan paroxysms every fourth day. Misprints were observed only on pages vii and 711.

ELLISTON FARRELL

*Science and Scientists in the Netherlands Indies*, by PIETER HONIG, Ph. D., Member of the Board for the Netherlands Indies, Surinam and Curacao; etc., and FRANS VERDOORN, Ph.D., Botanical Advisor to the Board for the Netherlands Indies, Surinam and Curacao; etc. 1945, New York City, Board for the Netherlands Indies, Surinam and Curacao.

In a series of original articles, reprints, biographical sketches and reviews which recount the development and present status of science in the Netherlands Indies, this volume presents a fascinating and authoritative picture of the Indonesian environment. Nearly all of the articles are in English and it is gratifying to have a record of the advancement of Dutch science in the Far East available to the many scientific workers unfamiliar with the Dutch tongue. Reports of immediate interest to physicians include a review by Snapper of the contributions made to medicine by Eijkman, Swellengrebel, Otten and other Dutch East Indies physicians, a report by Donath on the history of Dutch investigations of beri-beri and a series of chapters on the history of cinchona cultivation. Medical men will also be attracted by the biographical note by Professor Sirks on Rumphius, the blind seer of Amboina, the Indian Pliny, one of the grandest figures in the history of science, whose life story is a source of profound inspiration.

In summary, here is a book of great intrinsic interest which reveals to that large body of English and American scientists unable to read Dutch the substantial scientific achievements of Dutch Colonials in the Netherlands Indies. To the extent that scientific advancement is a political goal, this volume presents a strong argument for the restoration of Dutch Colonial rule.

ELLISTON FARRELL

# TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE, FORTY-FIRST ANNUAL MEETING

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*American Society of Parasitologists:* J. T. Culbertson.

*American Association for the Advancement of Science:* H. C. Clark, E. C. Faust.

*Division of Medical Sciences of the National Research Council:* H. E. Meleney (for a period of 3 years from July 1, 1944).

MEETING IN CINCINNATI, OHIO, IN CONJUNCTION WITH THE NATIONAL MALARIA SOCIETY AND THE AMERICAN ACADEMY OF TROPICAL MEDICINE, AS GUESTS OF THE SOUTHERN MEDICAL ASSOCIATION, NOVEMBER 12-15, 1945.

#### BUSINESS MEETINGS

##### THE MINUTES OF THE COUNCIL MEETING

The annual business meeting (which was preceded by the annual luncheon) of the Officers and Council of the Society was held at 12:30 p.m. on Tuesday, November 13, at the Gibson Hotel. Those present were: Doctors C. F. Craig, J. S. D'Antoni, R. E. Dyer, J. F. Kessel, O. R. McCoy, P. F. Russell, E. I. Salisbury, J. S. Simmons and R. B. Watson. Those absent were Doctors G. R. Callender, L. T. Coggeshall, E. G. Hakansson and A. J. Warren. The Society had the pleasure of having one of its former presidents, Dr. H. C. Clark, present at this time. The President, Dr. R. E. Dyer, presided.

1. The minutes of the previous meeting, November 14, 1944, were accepted as published in the March, 1945, issue of *THE AMERICAN JOURNAL OF TROPICAL MEDICINE*.

2. The president appointed Doctors McCoy and Watson as the Resolutions Committee.

3. The Secretary's report was presented as follows:

*Membership:* The total active membership of the Society as of November 13 is 1,309, as compared with 1,213 on approximately the same date in 1944. The increment and other changes are as follows:

8 deceased

4 resigned

19 delinquent in 1945

121 new members were added during the year and the names of 44 others will be proposed for Council approval at this meeting, making a total of 165 members elected in 1945.

2 Honorary members were elected during the year.

At the present time the Society has 4 Emeritus and 22 Honorary members. In addition (and in addition to subscriptions by libraries, institutions, etc.) 19 non members subscribe to *THE AMERICAN JOURNAL OF TROPICAL MEDICINE*. The Society thus carries a total of 1,398 names of individuals and organizations on its books at this time, as compared to 1,251 at the end of 1944.

At the present time the status of 8 members is completely unknown, including military men in this country and overseas and members in this country whose mail has not been returned and who have undoubtedly joined the Armed Forces without notification of their changes of address to the Secretary of the Society.

Certain details concerning the membership are of interest:

There are 627 non-military members, of whom 456 are within, and 171 outside of, the Continental United States.

There are 498 members in service, of whom 275 are stationed within, and 223 outside of, the Continental United States.

There are 173 members "in status quo," divided as follows: 17 (both military and non-military) are in areas in which mail service is still suspended; 9 are non-military delinquents for 3 years whose mail has been returned; and 37 are military delinquents for 3 years whose mail has been returned. The remaining 109 are members whose delinquency is less than 3 years, whose mail has been returned, and for whom no forwarding addresses are available.

The roster of new members approved during the year and the roster of members to be approved at this meeting appear immediately following this report. Those who resigned from active membership during 1945 are:

HIRAM J. BUSH  
ROLAND C. CONNOR

RENE J. DUBOS  
THOMAS B. TURNER

With regret the names of the following members are listed as having died within the past year:

W. N. BISPHAM  
ISRAEL J. KLIGLER  
OSWALD E. DENNEY  
• THOMAS MAXWELL

LAWRENCE GETZ  
RAYMOND C. SHANNON  
RICHARD G. HENDERSON  
CHARLES G. SINCLAIR

Captain Thomas Maxwell was killed in line of duty. As usual, letters of condolence were sent to the families of deceased members, as is the custom whenever the Secretary's office is notified or learns of the deaths.

*Circular Letters:* The following circular letters were sent out in 1945 to the Officers and Council:

March 13, containing information of the profit received from Williams & Wilkins in the amount of \$2,099.35 and announcing that the Office of Defense Transportation had curtailed all meetings in 1945 until further notice.

May 12, stating that the prospects of holding the annual meeting appeared better and asking suggestions for the program.

July 24, outlining plans, in the event that the annual meeting could not be held, to set a date for holding a Council meeting; to utilize papers sent in for the meeting in THE AMERICAN JOURNAL OF TROPICAL MEDICINE; and to confer the Bailey K. Ashford Award.

September 15, announcing that the annual meeting would be held in Cincinnati November 12-15, and that Colonel Paul F. Russell had been selected to deliver the Charles Franklin Craig Lecture.

October 2, giving as many details of the meeting as were then available and asking for suggestions for speakers on the program.

October 9, giving a tentative schedule of events for the meeting and commenting on the difficulties of preparing a program under the existing circumstances and with such brief notice.

October 26, announcing that Dr. James Watt had been nominated for the Bailey K. Ashford Award.

Circular letters listing the names of new members for Council approval were sent out February 7, June 4 and October 2.

*Action of the Council by Correspondence:*

(a) Approval of the names of 121 applicants for membership in the Society during 1945 (2/7, 6/4 and 10/2).

(b) Approval of changing the Council meeting at the annual meeting from Monday to Tuesday (10/2).

(c) Approval of defraying traveling expenses to the meeting of the Editor of THE AMERICAN JOURNAL OF TROPICAL MEDICINE, Colonel Charles F. Craig (10/26).

The term of the present Secretary expires with the 1945 meeting of the Society, and he may properly make certain comments on the business of the office. He has been fortunate, because of his personal office arrangements, in keeping the expenses of the Society at a minimum, but future secretaries may be less fortunate. It is therefore recommended that hereafter an allowance up to \$100 per month be allotted to the Secretary of the Society for office assistance, and that if he finds it impossible to operate the office within this limit, he ask for an increase of the sum by Council action. It is also recommended that in the future the traveling expenses to the annual meeting of both the Editor of THE JOURNAL and the Secretary of the Society be paid by the Society. The income of the Society warrants the expenditures proposed.

To simplify the transition in the Secretary's office and to facilitate the work of the Secretary to be elected at this meeting, the following proposals are made:

(a) That the outgoing Secretary send out bills for the coming year, making them payable to the incoming Secretary.

(b) That the outgoing Secretary be allowed a period of 20-30 days from the date of the meeting to clear up secretarial and financial matters presently under way.

(c) That the outgoing Secretary complete Volume 2 of *Tropical Medicine News* and that the financial account of the *News* be given to the incoming Secretary (the Secretary of the Society also being Editor of the *News*) after the close of the calendar year.

(d) That the incoming Secretary at once obtain quotations for publication of the *News* as well as contracts for advertising for 1946.

The Secretary again expresses his sincere appreciation of the cooperation shown to him by the Officers, Council and members of the Society during the past year. The brief advance notice of the annual meeting naturally made it impossible to execute all plans as efficiently as might be desired but thanks to the coopera-



tion of all concerned, a successful meeting was planned and held. Attention is again called to the excellent cooperation of the Southern Medical Association, through its Secretary-Manager, Mr. C. P. Loran, with this Society.

## ROSTER OF NEW MEMBERS APPROVED IN 1945

ABRAMSON, WILLIAM	HARVIE, FRED H.
ADAMSON, WILLIAM B.	HAUSHEER, WALTER C.
ADDIS, CLARENCE J.	HENTEL, WILLIAM
ADLER, DAVID L.	HERRERA, JULIO ROBERTO
ALVARADO, CARLOS A.	HOOVER, CARL H.
BAILLIE, JAMES H.	HUMPHREY, ARTHUR A.
BAKER, JOHN M.	HUNT, TALMAGE EVELYN
BALAMUTH, WILLIAM	INGHAM, GEORGE KENNETH
BARNETT, ROY N.	JACOBSON, FRANK J.
BATES, MARSTON	JARCHO, LEONARD W.
BAUER, FRANZ K.	JOHNSON, ELEANOR M.
BECKSTEAD, JAMES LEWIS	JOHNSTON, HAROLD M.
BEINAR, PETER JOSEPH	JORON, GUY ERNEST
BERLE, BEATRICE B.	KAUNG, DAVID TUN-WHE
BISTOWISH, JOSEPH M. JR.	KELLY, HOWARD GARFIELD
BLAND, C. BRINLEY	KIRN, JOSEPH D.
BRENNER, JOEL JEROME	KNIGHT, LEON A.
BUNTING, HENRY	KOLMER, GEORGE A. L.
BURNS, JOHN LLOYD	KUHN, FREDERICK L.
BUTLER, FRED ARTHUR	KULCSAR, DESIDER
CALABRESE, ARTHUR B.	KUNTZ, ROBERT E.
CALDWELL, JOHN WILLIAM	LADD, ARTHUR C.
CAMPBELL, FRANK C.	LAMAR, ROBERT F.
CHESNICK, REUBEN B.	LEVY, BERTRAM L.
CHIDSEY, ANDREW D. III	LIEBMANN, JAMES
COEN, WILLIAM B.	LILLY, CHARLES O'DOWD
CRESPO, JULIO A.	MC CREARY, THOMAS W.
DANIELS, CHARLES J.	McMORROW, CLYDE HENRY
DAO, LUIS	MAMULA, PETER S.
DARROW, EDITH M.	MARKELL, EDWARD K.
DIAZ, FERNAND	MEGIBOW, RAYMOND SAMUEL
DILLE, CHARLES A.	MORGAN, JULIA
DOWRIE, JAMES O.	MUGRAGE, EDWARD R.
DOYLE, FRANCIS E.	MUKROE, EUGENE G.
DUMANIS, ABRAHAM A.	MURRAY, THOMAS A.
EHRENREICH, THEODORE	MUSCHEL, LOUIS H.
ELORDUY, CARLOS CALERO	NEGhme, AMADOR RODRIGUEZ
FARISH, CLARENCE G.	NELSON, E. CLIFFORD
FEDER, AARON	NEWTON, WILLIAM A.
FLAIZ, THEODORE R.	NOEHREN, THEODORE HENRY
FORMAN, DOUGLAS N.	OLIVER, SAMUEL
GAGNON, BERNARD H.	PARE, J. PETER ARTHUR
GOLDBLOOM, A. ALLEN	PAUL, JEROME T.
GOLDMAN, LEON	PAYNE, EUGENE HAROLD
GUSTAFSON, CARL J.	PETERSON, CARL MELANCTON
HAMBLET, JOHN B.	POLLACK, DAVID
HART, THOMAS A.	POOLE, JOHN B.
HARTZ, PHILIP H.	ROGERS, MARY E.

RUDOLPH, LIONEL  
 SCHICK, ARMIN F.  
 SCHILDKROUT, HERMAN  
 SCHLEIFSTEIN, JOSEPH I.  
 SCHOFIELD, ADOLFO P.  
 SEASHORE, ROSEL THEODORE  
 SHAPIRO, LORNE  
 SHELDON, ALBERT JOHN  
 SHRADER, JACK COURTNEY  
 SLOAN, NORMAN R.  
 SMITH, HUGH H.  
 STOVER, IRWIN

SUN, CHIH JUNG  
 SUTTON, GERALD L.  
 TAINTER, MAURICE L.  
 TAVSEND, MILTON EDWIN  
 THETFORD, NORMAN D.  
 THOMSON, HENRY H.  
 TILLEMA, SIKKE  
 TOBIAS, NORMAN  
 TUNICK, ARTHUR M.  
 VERGARA, HUMBERTO  
 WISSEMAN, CHARLES L. JR.  
 WOO, THERESA T.

WOOLINGTON, SAM S.

Listed below are the new members who have been approved by the membership committee but upon which the Council action has not been taken:

ADVOCATE, SEYMOUR  
 ALLEN, RALPH F.  
 BAIRD, ELWOOD E.  
 BLUE, GORDON D.  
 BOLES, CLIFFORD R.  
 BOZARTH, CLYDE L.  
 BROSSAU, BERNARD L. P.  
 BURCHENAL, JOSEPH H.  
 COHEN, IRVIN JOSEPH  
 CONDIE, ROBERT S.  
 DAMMIN, GUSTAVE J.  
 DAVIS, MURRAY McCULLOCH  
 DONNELL, MARGARET M.  
 EILERT, MARY LOUISE  
 ELLIS, JOHN M.  
 EVANS, ALFRED S.  
 EVELAND, WARREN CHESTER  
 GILLESPIE, FRANK S.  
 GRADWOHL, RUTHERFORD B. H.  
 HARDY, ALBERT V.  
 HOWE, CLIFTON D.  
 JACHOWSKI, LEO ALBERT JR.

KERR, KATHEL B.  
 LAVERNE, ALBERT A. I.  
 MACKENZIE, SEYMOUR G.  
 MACLEOD, IAN MURRAY  
 MCGOEY, CHARLES J.  
 McNALLEY, NORMAN HENRY  
 MAREN, THOMAS H.  
 MATSON, JAMES R.  
 PIJOAN, MICHEL  
 PUGH, H. LAMONT  
 ROZOV, AURELIA  
 SABIN, ALBERT  
 SCHAPIRO, MARK M.  
 SHOOKHOFF, HOWARD B.  
 SIMMONS, SAMUEL W.  
 SINDERBRAND, ROBERT E.  
 STUBBS, TRAWICK H.  
 TOWN, FLOYD R.  
 TULLIS, JOHN L.  
 VAN DEN BERGHE, LOUIS S.  
 WEINSTEIN, PAUL P.  
 WELT, LOUIS G.

4. The following action was taken on the Secretary's report:

- (a) 44 new members were elected by the Council.
- (b) 19 members delinquent in dues were dropped from the rolls.
- (c) Resignations submitted by 4 members were accepted.
- (d) The deaths of 8 members were noted with deep regret.
- (e) The sum of \$100 monthly was voted to the Secretary for office expenses.
- (f) Allowance for traveling expenses of the Editor of THE AMERICAN JOURNAL OF TROPICAL MEDICINE and of the Secretary of the Society to the annual meeting of the Society was approved as a permanent annual expenditure.
- (g) The suggestions made by the retiring Secretary for the taking over of the office by the incoming Secretary were approved as made.
- (h) It was voted that all members of the Society presently "in status quo"

should remain in that category and not be dropped from the Society rolls, whether or not they were delinquent in dues and regardless of the cause of their unknown whereabouts. It was further voted that when any member in this category made contact with the Society in the future he would be permitted to renew his membership without payment of back dues unless he wished to receive back issues of THE AMERICAN JOURNAL OF TROPICAL MEDICINE, which (if they were available) would be supplied at the usual rate of \$5.00 per annum.

5. The report of the Treasurer was read (as follows) and accepted:

### CHECKING ACCOUNT

#### *Receipts*

Balance on hand, November 7, 1944.....	\$920.80	
Membership dues.....	5051.58	
Williams & Wilkins Company for profit on Volume 24 (1944) of THE JOURNAL.....	2099.35	
Checks not cashed by members (1944).....	6.34	
Refunds of exchange paid—year 1944.....	16.35	
Overpayments, refunds from publisher, etc.....	<u>61.10</u>	
Total.....		\$8155.52

#### *Disbursements*

To Charles F. Craig, Editor of THE JOURNAL for secretarial assistance.....	200.00	
To Secretary, J. S. D'Antoni, for secretarial assistance year 1945.....	583.00	
Postage.....	146.84	
Stationery and printing.....	73.69	
Telegrams and telephone calls.....	12.12	
Exchange paid bank on deposits for 1945.....	24.83	
Bank Charges.....	8.83	
Checks returned (not sufficient funds).....	10.00	
Refunds of overpayment for dues.....	24.76	
Extra copies of NEWS sent malaria units overseas.....	24.00	
Federal withholding tax paid.....	7.20	
U. S. War Savings Bonds—Series F 3—\$1000 (maturity value) —\$740.00 each.....	2220.00	
Williams & Wilkins Co., Publishers, for THE JOURNAL....	3963.34	
Incidental Expenses.....	<u>6.20</u>	
Total.....		\$7304.81
Balance on hand, November 7, 1945.....	<u>850.71</u>	<u>\$8155.52</u>

#### ASSETS

Balance in checking account.....	838.87	
Petty cash on hand.....	11.84	
U. S. War Savings Bonds—Series F*.....	4662.00	
Addressing machine (purchased 1944—cost).....	\$124.88	
Filing cabinet (purchased 1942—cost).....	<u>21.43</u>	<u>146.31</u>
		\$5659.02

\* The amount shown is the purchase price. The bonds will not mature for 12 years from date of purchase.

## LIABILITIES

Due Williams & Wilkins Company for subscriptions  
to Journal.....  
Net Assets.....

154.00  
\$5505.02

Doctors J. F. Kessel and P. F. Russell were appointed by the Chair to audit the Treasurer's books and to approve them if correct.

6. The report of the Editor of THE JOURNAL, Colonel Charles F. Craig, was presented as follows:

The Editor is pleased to report that with the issue of the November number of THE JOURNAL it will complete its twenty-fifth year with the largest circulation in its history, a circulation which will probably not be exceeded during the coming year as it is natural that with the demobilization of the thousands of physicians who have served during the war there will be a lessened interest in tropical medicine. It is believed, however, that this interest will be maintained to a large extent and that the circulation of THE JOURNAL will continue to be satisfactory.

With the signing of the new contract with the publishers, THE JOURNAL will continue to be a source of income to the Society, as it was during the year 1944, and it is a matter of satisfaction to the Editor that from a deficit of nearly \$8,000 when he became Editor, it is now a "paying institution," with the old debts settled and a substantial amount standing on the credit side of the ledger. This fortunate position is due to the earnest efforts of our secretaries in building up a larger subscription list and to the many contributors who have enabled us to publish such valuable papers upon subjects concerning tropical medicine. Our thanks are also due to the Rockefeller Foundation, which has paid for the publication of many most valuable and interesting papers contributed by its members, and to other institutions and authors who have paid similar expenses in whole or in part, thus enabling us greatly to exceed our page allowance and to add to the scientific value of THE JOURNAL.

During the past year it has been impossible for the publishers, by reason of manpower shortages, unskilled labor, and the necessity of fulfilling Government contracts, to publish THE JOURNAL on time, and we are thankful to our subscribers that this fact has been recognized and for their patience. At the present time the September number is almost ready for distribution and the proofs of the November number have been corrected and sent to the printer. It is therefore hoped that it will not be long before THE JOURNAL will again be published on schedule.

Because of the shortage of paper it was necessary to reduce the size of the type and to print THE JOURNAL in a double-column format during 1945. This change did not improve the appearance of THE JOURNAL, but it did enable us to print more papers in a smaller space. With the January, 1946, number, we will return to the former format, which employed a larger type and a single-column page.

Owing to the limitation on pages in THE JOURNAL long papers cannot be printed unless the author or the institute from which they come is willing to de-

fray the cost of excess pages. At the present time each author is allowed 6 pages and 2 full page plates, and the Editor would ask that authors bear this in mind in submitting papers for publication.

There has been a very satisfactory increase in clinical papers during 1945, many of them originating from the Army, Navy and Public Health Services. This has made THE JOURNAL of greater interest to clinicians, and the Editor hopes that clinical papers will continue to be submitted, as well as purely scientific contributions. He regrets that owing to the very large number of papers submitted during the past year, he was forced to return many valuable contributions because of lack of space, but it is most encouraging that this condition existed, as it demonstrates the greatly increased interest in tropical medicine and the good opinion held of THE JOURNAL by those who contributed the papers. This situation could be corrected by publishing THE JOURNAL every month, but the Editor does not believe that the time is yet ripe for such a venture by the Society. It is believed that we should wait until reconversion is completed before attempting monthly publication, for until that time one will not be certain of the effect upon the subscription list of the termination of the war and the probable loss of interest in tropical medicine by many physicians who are now subscribers and members of the Society.

In closing, the Editor desires to express to the Secretary and other officers of the Society, and to the authors who have submitted papers to THE JOURNAL, his thanks and appreciation for their support and patience during the trying times of the past year. Despite the delay in publication, there have been very few complaints and he desires to express his appreciation of the support that has been given him by the members of the Society throughout the year.

7. The following action was taken on the Editor's report:

(a) It was received with a special vote of thanks for Colonel Craig's valuable work.

(b) The sum of \$200 was voted to the Editor for secretarial assistance during 1946.

8. The report of the Editor of *Tropical Medicine News* (Dr. Joseph S. D'Antoni) was presented as follows:

With the appearance of the December, 1945, issue (which will not appear until January, 1946, because of delay in the publication of the October, 1945, issue), *Tropical Medicine News* will have completed its second year. All issues of Volume 2 were published in New Orleans by the Busy Printing Company. Arrangements with this company have been most satisfactory. The charges are minimum (\$154.50 for 1,500 copies of each issue), the publication is mailed out within 3 to 4 weeks after copy is delivered, and cooperation is excellent.

The Editor is happy to report that during its second year of existence *Tropical Medicine News* continued to serve the Society as an up-to-the-minute news bulletin, as evidenced by numerous requests to be placed on the mailing list by libraries, pharmaceutical houses, and Federal and state health departments. In addition, 200 copies of each issue are sent, without cost to the Society, to Lieu-

tenant Colonel Oliver McCoy of the Division of Preventive Medicine, Office of the Surgeon General, whence they are distributed to malaria units stationed overseas. The mailing list of the *News* for the October issue totaled 1,385 copies.

Advertising space was requested during the year by the Winthrop Chemical Company, Inc., and Merck & Co. Previously all advertising space has been pre-empted by G. D. Searle & Co., Wyeth, Inc., and Eli Lilly & Co. An arrangement has been worked out to divide the advertising space in the 6 issues per year among these 5 firms.

As reported in various issues of the *News* during 1945, a contest is under way to provide a new cover for the bulletin, to replace the present greatly discussed cover. The 3 designs submitted in this contest, together with the present cover, will be exhibited and voted on (by number) at the hospitality sessions. The winner of the contest is to receive as award the sum of \$25.00, in the form of remission of dues to the Society for 5 years and subscription to *THE AMERICAN JOURNAL OF TROPICAL MEDICINE* for the same period. The outcome of the contest will be reported in the *News*.

As mentioned in the Secretary's report, it is recommended that the present Editor or the *News* publish the December issue, after which material for future issues, as well as all accounts and files, be forwarded to the new Secretary-Editor.

Attention is again called to the fact that the *News* can be successfully edited by the Secretary of the Society only if a constant flow of material of interest to the membership is regularly available. No editor can of himself publish an interesting bulletin. The cooperation of the whole membership is essential if the *News* is to continue to flourish and properly to represent the American Society of Tropical Medicine as its official bulletin.

The difficulties in issuing the October number of the *News* illustrates what lack of cooperation can be responsible for. Because of insufficient material publication was delayed for 5 weeks. As a result, even though the printer by overtime work rushed the issue through and produced it in 2 weeks, instead of in the usual 3-4 weeks, the membership was not adequately informed that the annual meeting would be held. As a further result, many members will not attend the meeting, either, because they had no notice of it, or because they could not secure hotel accommodations, all rooms being completely sold out 3 weeks before the meeting. If material had been available, the October issue could have been published in advance of the regular publication date, and full information concerning the hastily scheduled meeting could have reached the members in time for them to act on it.

Furthermore, members should feel responsible for sending in news items about themselves and their associates. The Editor is aware that many persons feel a certain bashfulness in this respect. Actually, their cooperation is desired and appreciated by the readers of the *News*, and greatly lightens the work of the Editor. It is hoped that the new Editor will have the full cooperation of the membership in this regard.

9. The report of the Editor of the *News* was accepted with special commendation.

In addition, the financial report for *Tropical Medicine News* for 1944 was accepted as published in the February, 1945, issue, and the estimated financial report for the calendar year of 1945 was heard.

10. The following committee reports were presented:

(a) *The Committee on Honorary Membership.* The name of Major General Gordon Covell was presented from the floor, and the nomination was accepted. It was voted that the names presented by this committee be forwarded to the incoming committee for consideration.

(b) *The Membership Committee.* This report is included in the report of the Secretary (section 4a).

(c) *The Walter Reed Medal Committee.* Members of this Committee were notified that this award will be available in 1946.

(d) *The Charles F. Craig Lecture Committee.* The selection of Colonel Paul F. Russell to present this lecture was noted.

(e) *The Program Committee.* (For the complete program see pp. 372-377)

(f) *The Committee on War and Post-War Tropical Medicine.* This Committee proposed the following resolutions:

1. That the Society encourage the biological laboratories to make certain diagnostic antigens commercially available, in order to assist physicians at large in the diagnosis of tropical diseases. The list tentatively might include the antigens for echinococcus, schistosomiasis, coccidioides, filariasis, and possibly *Endamoeba histolytica* antigens for complement fixation.

2. That the Secretary of the Society be instructed to notify the Veterans Administration of the past action of the Society and the resulting opportunities for the training of personnel in tropical diseases at the Public Health Services Center in Atlanta, Georgia.

3. That the Secretary of the Society be instructed to inform the Public Health Services Training Center in Atlanta, Georgia, that it is the opinion of the Society that introduction of *Ancylostoma duodenale* by returning military personnel is a definite possibility, and that the Training Center take suitable steps for the dissemination of this and other pertinent information concerning infection with this organism to public health agencies and practising physicians.

4. That the Secretary of the American Society of Tropical Medicine be instructed to send the following letter to the President of the United States:

The wartime experience of the United States during the last few years has emphasized the importance of tropical medicine and the control of tropical diseases, not only to the Medical Departments of our Army, Navy and Public Health Service, but to the entire civilian medical profession of the country. The protection of American citizens against tropical diseases in the future will depend on the provision of adequate facilities for training our physicians in tropical medicine and for research on tropical diseases.

The opportunities for such training and research are now woefully inadequate within the boundaries of the United States. This fact was evidenced by the lack of sufficient personnel experienced in the control of tropical diseases to meet the needs of the armed forces at the beginning of the war. Because of this deficiency

the Army and Navy were forced to establish special emergency educational programs in order that their personnel might at least have some elementary training in the diagnosis, treatment and prevention of these dangerous illnesses.

The American Society of Tropical Medicine is greatly concerned with the fear that this essential phase of post-war American medicine may be allowed to deteriorate to its pre-war level. Therefore, the Society earnestly and strongly recommends that the Government of the United States provide sanction and support of an arrangement designed to afford facilities in a place or places in suitable areas of the tropics for training in the tropical diseases and where research in this specific field may be accomplished. Specifically, it is recommended that funds be provided and that arrangements be made with other governments for the immediate and active development of this important phase of American medicine.

The members of the American Society of Tropical Medicine will be glad to assist in making specific plans for the achievement of this objective.

The name of this Committee was by vote changed to the Committee on Post-War Tropical Medicine.

(g) *Bailey K. Ashford Award Committee.* The report of this Committee is included in the report of the Secretary (section 3). The Secretary of the Society was informed by the Eli Lilly Company, donors of the Award, that it would be available on three additional occasions, that is, every second year for the next 6 years.

(h) *The Committee of the Teaching of Tropical Medicine.* This report, which was prepared for the Chairman, Dr. H. E. Meleney, will appear in a forthcoming issue of *Tropical Medicine News*.

(i) *The Resolutions Committee.* It was recommended to the Society that letters of appreciation be sent to the following individuals and organizations: Mr. C. P. Loranz, Secretary-Manager of the Southern Medical Association; Colonel Paul F. Russell, Charles Franklin Craig Lecturer for 1945; the Hotel Gibson; the Racquet Club of Cincinnati; the Kenton-Campbell Medical Society; Dr. Joseph S. D'Antoni, retiring Secretary-Treasurer of the Society.

11. The report of the Society representative to the American Foundation for Tropical Medicine was reported verbally by Dr. E. I. Salisbury, who summarized the meetings held by the Foundation during the year, upon which he had been in attendance. Dr. Salisbury was appointed representative to the Foundation for 1945-1946.

The American Society of Parasitologists had had no meeting during the year, and the Society representative to it therefore had no report. The same was true of the American Association for the Advancement of Science.

12. It was voted to continue affiliations with the Southern Medical Association and the National Malaria Society until this action should be rescinded by the Council.

13. It was voted that the Secretary of the American Society of Tropical Medicine and the Secretary of the National Malaria Society make all possible efforts to prevent conflicts of simultaneous scientific sessions at future meetings of these organizations.



14. The Council voted to propose the following ballot of new officers to the Society: President-Elect, Edward I. Salisbury; Vice-President, Joseph S. D'Antoni; Secretary-Treasurer (for 3 years), Norman H. Topping; Council for 4 years, Justin Andrews and Milford E. Barnes, and for 3 years, to fill the unexpired term of Edward I. Salisbury, Paul F. Russell.

15. Appointments were made by the Council to the Committee on the Teaching of Tropical Medicine, the Walter Reed Medal Committee, and to the Committee on Post-War Tropical Medicine. These appointments, and the appointments made by the President, appear on pages 1-2.

16. New Business:

(a) The meeting heard various letters concerning the Secretary's office dealing with routine matters.

(b) The following resolution was received from the American Academy of Tropical Medicine:

WHEREAS, the exigencies of recent years have intensified research on problems in the field of tropical medicine, resulting in numerous and significant discoveries which materially contributed to the success of our military operations in foreign theaters, and

WHEREAS, since the general interest in tropical medicine is more widespread than at any time in the past, and the medical profession is keenly aware of these problems which become more pressing because of the phenomenal growth of international air travel, and

WHEREAS, it is important that information of these discoveries be brought to the world in order that with the return of peace the benefits of this knowledge may be made available to promote the well-being of the people of the world, and

WHEREAS, an international reunion of those who have contributed to these advances with others who may be qualified to disseminate their benefits is the most effective measure to generally diffuse this knowledge, and since such an international gathering of scientists will materially contribute to the better international understanding and cooperation so essential to an enduring peace,

BE IT RESOLVED, that the American Society of Tropical Medicine heartily endorse the proposal of the American Academy of Tropical Medicine that an international congress on tropical medicine and malaria be held in the United States at an early date, and that the American Society of Tropical Medicine join with the American Academy of Tropical Medicine, in petitioning the State Department of the United States government to officially sponsor, and invite international participation in such a gathering at as early a date as is regarded as opportune, and direct the secretary of the American Society of Tropical Medicine to formally advise the State Department of this endorsement,

AND BE IT FURTHER RESOLVED, that the President of the American Society of Tropical Medicine is authorized and empowered to appoint a member of the Officers and Councilors of the American Society of Tropical Medicine to represent the American Society of Tropical Medicine on a committee composed of duly authorized and appointed representatives of the

American Academy of Tropical Medicine

American Society of Tropical Medicine

National Malaria Society

American Society of Parasitologists

Southern Medical Association

American Medical Association

American College of Physicians

American Association for the Advancement of Science, and the

Section on Medical Science of the National Research Council,

to meet on call from the president of the American Academy of Tropical Medicine, for organization, and in their organized capacity to assist the State Department in developing, promoting and holding such a congress, and the American Society of Tropical Medicine will further give all practicable support to the realization of this project.

17. Action: (a) This resolution was accepted.

(b) Colonel Paul F. Russell was selected to represent the Society.

## MINUTES OF THE ANNUAL BUSINESS MEETING

NOVEMBER 15, 1945, 12:00 NOON

1. The minutes of the 1944 annual business meeting were accepted as published.

2. The transactions of the Council as set forth in items 1, 3, 4, 5, 6, 7, 8, 9, 10a, 10f, 10i, 12, 14 and 17 were approved.

3. Adjournment followed.

## SCIENTIFIC SESSIONS

The first scientific session of the Society was called to order at 9:00 a.m. Tuesday, November 13, in the Ballroom of the Hotel Gibson, by President Rolla E. Dyer, Washington, D. C. The program follows:

1. A report of the activities of the Distributing Center for Parasitological Specimens, 1943-1945, by George W. Hunter, III, Major, Sanitary Corps, Army Medical School, Washington, D. C. No discussion.

2. The tropical disease education program of the U. S. Public Health Service, by William S. Boyd, Trawick H. Stubbs and Paul P. Weinstein, U. S. Public Health Service, Atlanta. Presented by Doctor Boyd. Discussed by Doctors Barnes, Faust and McCoy.

3. Opportunities for training and research in tropical medicine and public health in Mexico, by Manuel Martinez Baez, Salubridad & Asistencia, Mexico, and E. Harold Hinman, Institute of Inter-American Affairs, Division of Health and Sanitation, Mexico. Presented by Doctor Hinman. Discussed by Doctor Cort.

4. Precautions by the Army to prevent the introduction of tropical diseases, by O. R. McCoy, Lieutenant Colonel, Medical Corps, AUS, Office of the Surgeon General, Washington, D. C. Discussed by Doctors Barnes, Craig, Faust, Kessel and Young.

5. The Tenth Charles Franklin Craig Lecture on Tropical Medicine: Lessons

in Malariology from World War II, by Paul F. Russell, Colonel, Medical Corps, AUS, Army Medical Center, Washington, D. C. No discussion.

6. Malarial problems of the British Army, by F. S. Gillespie, Colonel, RAMC, British Medical Liaison Officer with U. S. Army, Carlisle Barracks. No discussion.

7. The transmission of foreign *vivax* malaria relapsing in returned troops to neurosyphilitic patients, by Martin D. Young, John M. Ellis and Trawick H. Stubbs, U. S. Public Health Service, Columbia. Presented by Doctor Stubbs. Discussed by Doctor Young.

8. Methods for the study of insect repellents, by L. A. Jachowski, Jr., Lieutenant (jg) (H) USNR, and M. Pijoan, Lieutenant, Medical Corps, USNR, National Naval Medical Center, Bethesda. Presented by Lieutenant Jachowski. Discussed by Doctors Beckman, Fallis, Gillespie, Hertig and Pijoan.

### JOINT SESSION WITH THE NATIONAL MALARIA SOCIETY

The second scientific session of the Society was called to order at 9:00 a.m. Wednesday, November 14, in the Roof Garden of the Hotel Gibson, with Mr. H. A. Johnson, President of the National Malaria Society, and Doctor Rolla E. Dyer, President of the American Society of Tropical Medicine, presiding. The program follows:

9. Studies on imported malarias: II. Ability of California Anophelines to transmit malarias to foreign origin and other considerations, by Joseph A. Moore, Martin D. Young, Trawick H. Stubbs and Newton F. Hardman, U. S. Public Health Service, Malaria Research Laboratory, Columbia. Presented by Doctor Young. Discussed by Doctor Boyd.

10. The *Anopheles Gambiae* problem in Brazil and West Africa, 1941-1944, by Elliston Farrell, Major, Medical Corps, AUS, AAF School of Aviation Medicine, Randolph Field. Discussed by Doctors Russell, Kumm and Simmons.

11. The suppressive effect of NIH-204 and atabrine against sporozoite-induced *vivax* malaria (St. Elizabeth Strain), by G. Robert Coatney, W. Clark Cooper, Martin D. Young and Robert E. Burgess, National Institute of Health, Bethesda and Columbia. Presented by Doctor Cooper.

12. The protective and therapeutic effect of quinine sulfate against sporozoite-induced *vivax* malaria (St. Elizabeth Strain), by G. Robert Coatney, David S. Ruhe, W. Clark Cooper, Edward S. Josephson, Martin D. Young, and Robert E. Burgess, National Institute of Health, Bethesda and Columbia. Presented by Doctor Ruhe.

13. The course of the complement-fixation in fifty-three patients with sporozoite-induced *vivax* malaria (St. Elizabeth Strain), by Charles R. Rees, Samuel C. Bukantz, John F. Kent, G. Robert Coatney, W. Clark Cooper and David S. Ruhe, Army Medical School, Washington, D. C., and National Institute of Health Bethesda and Atlanta. Presented by Doctor Kent.

The three preceding papers were discussed by Doctor McCoy.

14. 2-Metanilamido-5-Chloropyrimidine, a potent agent in the prevention and suppression of avian malarias, by Emanuel Waletzky and Sterling Brackett, Research Laboratories of the American Cyanamid Company, Stamford. Presented by Doctor Waletzky. Discussed by Doctor Cooper.

15. Blood oxygen in ducks with malarial parasites, by R. H. Rigdon and H. H. Rostorfer, University of Arkansas School of Medicine, Little Rock. Presented by Doctor Rigdon. Discussed by Doctor Rostorfer.

16. Effect of certain nutritional deficiencies on avian malaria, by Albert O. Seeler and Walther H. Ott, Merck Institute for Therapeutic Research, Rahway. Presented by Doctor Ott.

17. Effects of quinine on Saurian malarial parasites, by Paul E. Thompson, Mary Imogene Bassett Hospital, Cooperstown. Discussed by Doctors Ackert, Brooke and Waletzky.

18. Studies on *Plasmodium cynomolgi* in Rhesus monkeys, by Fruma Wolfson and Mary Whitehead, Department of Protozoology, Johns Hopkins School of Hygiene and Public Health, Baltimore. (By title).

19. Toxic reactions following the administration of quinacrine hydrochloride, by Paul K. Smith, Major, AC, Chief of Department of Pharmacology, AAF School of Aviation Medicine, Randolph Field. Presented by Doctor Smith. Discussed by Doctor Farrell.

Discussion opened by Fratis L. Duff, Colonel, Medical Corps, AUS.

The third scientific session was called to order Wednesday afternoon, November 14, at 2:00 p.m., in the Roof Garden of the Hotel Gibson, by President Rolla E. Dyer. The program follows:

20. Heat rash as a problem in the Naval Service, by Gerald J. Duffner, Lieutenant, Medical Corps, USN, National Naval Medical Center, Bethesda. Discussed by Doctor Farrell.

21. The activity of the heteropolar cationic antiseptics against cysts of *Endamoeba histolytica*, by John F. Kessel and Frederick J. Moore, University of Southern California School of Medicine, Los Angeles. Presented by Doctor Kessel. No discussion.

22. Pathologic studies in monkey amebiasis, by Victor P. Bond, Warren Bostick, Eder Lindsay Hansen and Hamilton H. Anderson, University of California Medical School, San Francisco. Presented by Doctor Anderson. Discussed by Doctors Brooke, Clark, D'Antoni, Faust, Kessel and van den Berghe.

23. Preliminary report on the evaluation of penicillin in the treatment of Yaws, by James H. Dwinelle, Lieutenant Colonel, Medical Corps, AUS, Charles R. Rein, Lieutenant Colonel, Medical Corps, AUS, Thomas H. Sternberg, Lieutenant Colonel, Medical Corps, AUS, and Albert J. Sheldon, Major, Medical Corps, AUS, Office of inter-American Affairs, Army Medical Center, and office of the Surgeon General, Washington, D. C. Presented by Major Sheldon. No discussion.

24. Presentation of the Bailey K. Ashford Award to Doctor James Watt. Award was accepted for Doctor Watt by Doctor R. E. Dyer.

25. Tsutsugamushi disease (Scrub or mite-borne typhus) in the Philippine Islands during American re-occupation in 1944-1945, by Theodore E. Woodward, Major, Medical Corps, Cornelius B. Philip, Lieutenant Colonel, Sanitary Corps, and Ralph R. Sullivan, Lieutenant Colonel, Medical Corps, United States

of America Typhus Commission and Eighth Army. Presented by Major Woodward. No discussion.

26. Observations on the etiology and clinical features of Tsutsugamushi disease (scrub typhus) in New Guinea, by Francis G. Blake, Yale University School of Medicine, New Haven. Discussed by Doctors Bercovitz, Craig, Otto and Topping.

27. Azotemia in typhus fever, by A. Yeomans, Lieutenant Commander, Medical Corps, USNR, J. C. Snyder, Lieutenant Colonel, Medical Corps, USA, E. S. Murray, Lieutenant Colonel, Medical Corps, USA, R. S. Ecke, Major, Medical Corps, USA and C. J. Zarafonetis, Major, Medical Corps, USA, United States of America Typhus Commission, Washington, D. C. Presented by Lieutenant Commander Yeomans. No discussion.

28. Cutaneous Diphtheria: Report of 140 cases, by Clarence S. Livingood, Major, Medical Corps, AUS, James S. Forrester, Lieutenant Colonel, Medical Corps, AUS, and Daniel Perry, Captain, Medical Corps, AUS, Office of the Surgeon General, Washington, D. C. Presented by Major Livingood.

29. Neurological complications of cutaneous diphtheria, by Herbert S. Gaskill, Major, Medical Corps, AUS, and Milton Korb, Captain, Medical Corps, AUS, Office of the Surgeon General, Washington, D. C. Presented by Major Gaskill.

30. Myocardial complications of cutaneous diphtheria, by Calvin F. Kay, Major, Medical Corps, AUS, and Clarence S. Livingood, Major, Medical Corps, AUS, Office of the Surgeon General, Washington, D. C. Presented by Major Kay.

The three preceding papers were discussed by Doctors Kessel and Stilwell.

The fourth scientific session was called to order Thursday morning, November 15, at 9:00 a.m., in Parlors N, O, P and Q of the Hotel Gibson, with President-Elect James S. Simmons, Washington, D. C. presiding. The program follows:

31. The development of *Litomosoides carinii*, Filariid parasite of the cotton rat, *Sigmodon hispidus litoralis*, in the tropical rat mite, *Liponyssus bacoti*, by Roger W. Williams, Lieutenant (jg), USNR, and Harold W. Brown, School of Public Health, Columbia University, New York. Presented by Lieutenant Williams. Discussed by Doctors Porter, Sandground and Young.

32. A laboratory infection of the rat with filarial worms, by J. Allen Scott and Joy B. Cross, University of Texas School of Medicine, Galveston. Presented by Doctor Scott. Discussed by Doctors Kessel and Warren.

33. Studies on the specificity of intradermal tests in the diagnosis of filariasis, by Donald L. Augustine, Harvard Medical School, Boston, and Camille Lherisson, Medical School, University of Haiti, Haiti. Presented by Doctor Augustine. Discussed by Doctor Warren.

34. Serological relationships between antigenic extracts of *Wuchereria bancrofti* and *Dirofilaria immitis*, by Virginia G. Warren, Joel Warren, First Lieutenant, Sanitary Corps, AUS, and George W. Hunter, III, Major, Sanitary Corps, AUS, Army Medical Center, Washington, D. C. Presented by Doctor Virginia G. Warren. No discussion.

35. Chemotherapy of human filariasis (*Wuchereria bancrofti*) by administration of antimony compounds, by James T. Culbertson, Harry M. Rose, Federico Hernandez Morales, José Oliver Gonzalez and Caroline Pratt, Columbia University, New York, and School of Tropical Medicine, San Juan, P. R. Presented by Doctor Culbertson. Discussed by Doctors Brown, Faust and Martin.

36. A comparative study of the clinical and pathological pictures in *Schistosoma mansoni* and *S. japonicum* infections, by Louis van den Berghe, Institute of Tropical Medicine, Antwerp, Belgium. No discussion.

37. The diagnosis of Schistosomiasis Japonica: I. The symptoms, signs and physical findings characteristic of Schistosomiasis Japonica in a native family on Mindanao, Philippine Islands, by Ernest Carroll Faust, Tulane University School of Medicine, New Orleans, Willard H. Wright, U. S. Public Health Service, Bethesda, and Donald B. McMullen, University of Oklahoma School of Medicine, Oklahoma City. (Commission on Schistosomiasis, Army Epidemiological Board). Presented by Doctor Faust. Discussed by Doctors Brooke and Martin.

38. Blood levels of antimony in persons receiving tribalent and pentavalent antimonials, by G. F. Otto, T. H. Maren and H. W. Brown, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, and School of Public Health, Columbia University, New York. Presented by Doctor Otto. No discussion.

39. Infectivity of Pacific Island *Wuchereria bancrofti* to mosquitoes of the United States, by Don E. Eyles, U. S. Public Health Service, Harry Most, Major, Medical Corps, AUS, and Martin D. Young, U. S. Public Health Service, Moore General Hospital, Swannanoa. Presented by Doctor Eyles. Discussed by Doctors Brenner, Martin and Young.

40. Tolerance of fowls to moderate infections of ascarids and tapeworms, by J. E. Ackert and C. L. Wisseman, Jr., Kansas State College, Manhattan, and Southwestern Foundation Medical College, Dallas. Presented by Doctor Ackert. No discussion.

41. An attempt by feeding to induce in animals reactivity to *Trichinella spiralis* in the absence of infection, by G. T. Harrell, John Avera and Ellard Yow, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem. Presented by Dr. Avera. No discussion.

The following papers were listed by title or were not presented because of the essayists' inability to be present at the meeting:

42. The geographical distribution and laboratory diagnosis of *Salmonella* infections, by Oscar Felsenfeld and Viola Mae Young, Mount Sinai Medical Research Foundation, Chicago.

43. The treatment of human filariasis (*Wuchereria bancrofti*) by administration of melarsen oxide, by Harry M. Rose and James T. Culbertson, Columbia University, New York.

44. The diagnosis of Schistosomiasis Japonica: II. The diagnostic characteristics of the eggs of the etiologic agent, *Schistosoma japonicum*, by Ernest Carroll

Faust, Tulane University School of Medicine, New Orleans. (Commission on Schistosomiasis, Army Epidemiological Board).

45. Chemotherapy of Schistosomiasis by the administration of Urea Stibamine (Squibb), by Federico Hernandez Morales, José Oliver Gonzalez and Caroline Pratt, School of Tropical Medicine, San Juan, P. R.

### OTHER EVENTS

1. The annual luncheon of the Society was held Wednesday, November 14, at noon. The President of the Society, Dr. R. E. Dyer, Washington, D. C., who was introduced by the President-Elect, Brigadier General James S. Simmons, presented as his presidential address "Medical research in the post-war period." Former presidents were seated at the speaker's table, as were representatives from the Academy of Tropical Medicine and the National Malaria Society.

2. Well attended hospitality group sessions were held on November 12, 13 and 14 at 5:00 p.m. Informal talks were made at these sessions by Dr. C. Larrimore, who spoke on the education of the GI in personal hygiene against tropical diseases, and by Dr. E. C. Faust, who discussed his trip to Leyte as head of the Commission on Schistosomiasis.

3. The American Academy of Tropical Medicine held its twelfth annual dinner at 7:00 p.m. Wednesday, November 14, to which all members of the Society were invited. Dr. E. V. Cowdry was toastmaster.

Dr. Mark F. Boyd, Tallahassee, Florida, presented as his presidential address "International appraisal of tropical medicine."





# EXPERIMENTAL STUDIES OF BULLIS FEVER AND DENGUE FEVER

H. R. LIVESAY,<sup>1</sup> D. J. WILSON,<sup>2</sup> M. POLLARD<sup>3</sup> AND J. C. WOODLAND<sup>4</sup>

*From the Eighth Service Command Laboratory, Fort Sam Houston, Texas and Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas*

Since the syndrome of Bullis fever was originally described (1), there have been rather persistent informal opinions that this disease is essentially one of the known prevalent diseases. This was based on some similarity either in clinical manifestation or in etiological agent. Thus far, no relationship has been demonstrated between Bullis fever and the rickettsial diseases such as typhus, Q fever, and Rocky Mountain spotted fever (2, 3, 4) although they are all associated with arthropods and the Bullis fever agent has been described as rickettsia-like in morphology. No relationship has been demonstrated between Bullis fever and Colorado tick fever (5); although these two diseases are associated with ticks, fatalities are relatively rare, and both are characterized by the development of a marked leucopenia in the course of the disease.

The syndrome of Bullis fever exhibits a clinical similarity to dengue fever, only to the extent of manifesting a leucopenia. Both are associated with arthropods, and have a very low fatality rate. It was considered of importance, however, that the two diseases be studied immunologically in order that any possible relationships might be clarified. Since laboratory animals are not very susceptible either to Bullis fever or to dengue fever, the studies were confined to inoculation experiments in human volunteers.

## EXPERIMENTAL INOCULATIONS

Three healthy male volunteers were selected for inoculation with the Bullis fever agent (Chart I). Two of the volunteers (I and III) were inoculated with the yolk sac propagated tick strain of Bullis fever agent (5)—1.0 cc. subcutaneously. One volunteer (II) was inoculated with whole blood from a natural case of the disease—2.0 cc. subcutaneously. The latter inoculum had been stored in the dry ice chamber for five months after collection. It was then thawed and immediately inoculated.

On the fourth day after the inoculation all three of the volunteers developed symptoms of Bullis fever: generalized malaise, leucopenia, headache, generalized lymphadenopathy, and a mild fever. Case I had been inoculated two months previously with normal chick embryo yolk sac, from which no effect was manifested. Subsequent challenge with the Bullis fever agent indicated that there was no immunity induced to it by the normal yolk sac. These symptoms, induced by the Bullis fever agent, are similar to many of the naturally occurring cases of the disease, and had been previously reproduced, with minor modifica-

<sup>1</sup> Colonel, M.C., U.S.A.

<sup>2</sup> Major, M.C., U.S.A.

<sup>3</sup> Major, V.C., U.S.A.

<sup>4</sup> Colonel, M.C., U.S.A.

tions, in twelve volunteers both with whole blood inoculations (1.0 cc. subcutaneously) and with chick embryo yolk sac propagated material of human and of tick origin. This syndrome was considered to be consistent with that of the naturally occurring disease.

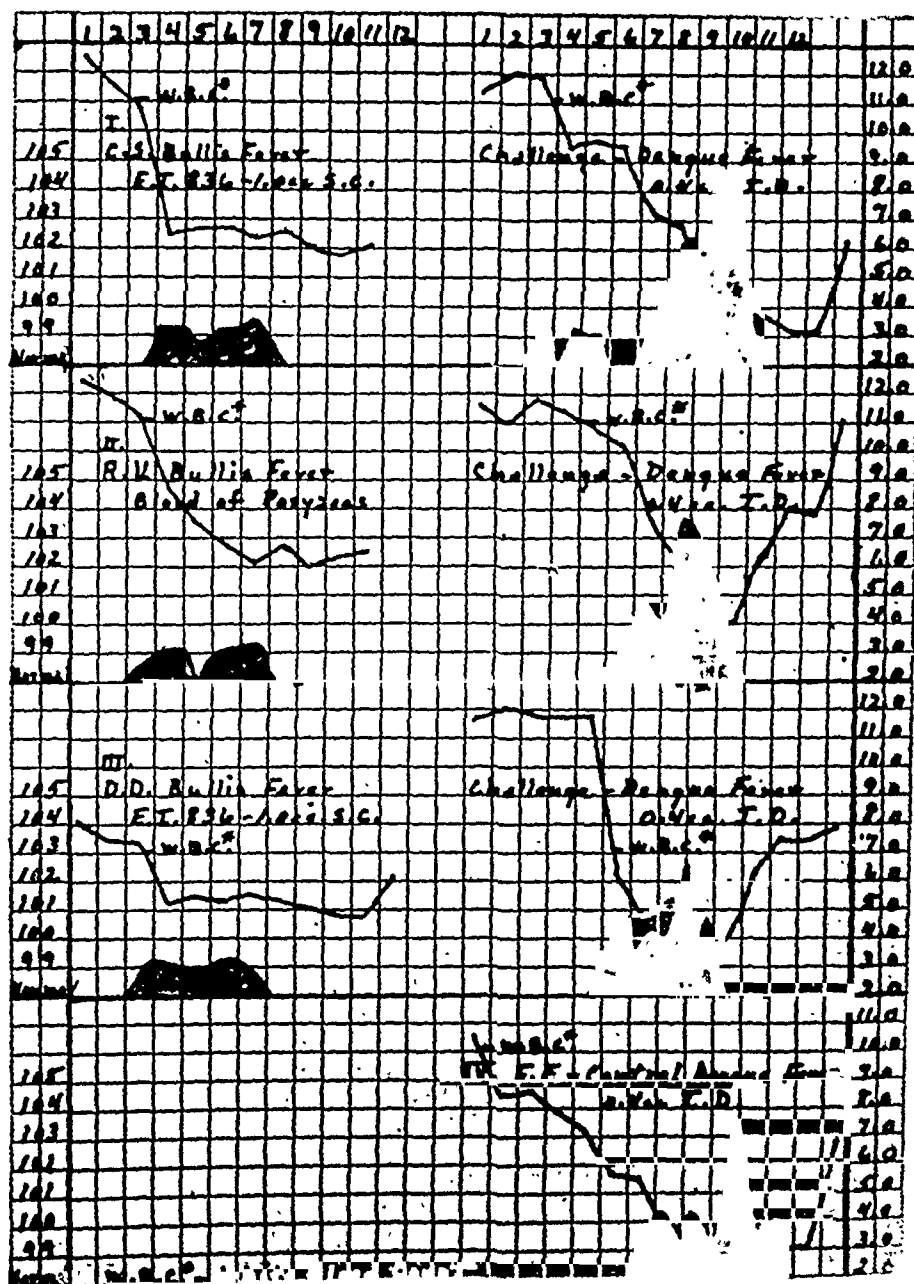


CHART I

Twenty-seven days after the defervescence of Cases I, II, and III, each was inoculated intradermally with 0.4 cc. of dengue-infected human serum.<sup>5</sup> In

<sup>5</sup> We are indebted to Lt. Col. A. B. Sabin, M. C. for the strain of dengue fever employed.

addition, one normal control volunteer was similarly inoculated with the dengue fever material. A typical syndrome of dengue fever developed on the sixth and seventh day after inoculation in all four of the volunteers. This was characterized by generalized malaise, headache, leucopenia, and generalized rash.

#### DISCUSSION

The temperature reactions which were induced with the Bullis fever material are not as high as are manifested by some of the naturally occurring cases; however, selected later cases have demonstrated a resistance to challenge inoculations with the chick embryo propagated material. This fact and the coincident leucopenia, malaise and lymphadenopathy serve to support the significance of the febrile reactions which were induced in the experimental cases.

On the basis of this challenge experiment, no relationship between Bullis fever and dengue fever could be demonstrated. It is possible that strain variations may alter the results to some extent; however, the evidence herein reported serves to differentiate the two diseases. This information serves to further delineate the clinical syndrome of Bullis fever from other known diseases which might appear to bear some resemblance to it.

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# THE RESIDUAL SPRAYING OF DWELLINGS WITH DDT IN THE CONTROL OF MALARIA TRANSMISSION IN PANAMA, WITH SPECIAL REFERENCE TO ANOPHELES ALBIMANUS<sup>1</sup>

HAROLD TRAPIDO<sup>2</sup>

*From Tropical Disease Control Section, Office of The Surgeon General, and  
The Gorgas Memorial Laboratory, Panama, Republica de Panama*

## INTRODUCTION

Since the discovery of the transmission of human malaria by anopheline mosquitoes, the most widely applied methods of mosquito control, other than naturalistic methods, have been those of draining or larviciding breeding areas and screening dwellings. Another technique which has been long known but less widely applied has been that of destroying adult female mosquitoes found in dwellings. Orenstein (1913) records that as early as 1908 the daily capture of mosquitoes in dwellings was employed as prophylactic measure against malaria in the Canal Zone. The work of Russell and Knipe (1939, 1940, 1941) and Russell, Knipe, and Sitapathy (1943), in India, and the earlier work of De Meillon (1936), in South Africa, has recently served to focus attention on this method. These authors used pyrethrum sprays rather than hand catching.

Where the malaria vector species involved is a domestic one the value of such a method is at once apparent. Since the "seed bed" of malaria is in the human population, the killing of the very mosquitoes which feed on this population is obviously the most direct means of attack on the transmission of malaria. The method is in effect a further refinement of "species control" whereby selective destruction of potentially infected mosquitoes is attained.

In India, Russell, Knipe and Sitapathy dealt with anopheline vector species which were house-resting during the day. Using various pyrethrum sprays, and spraying at intervals of three to ten days, they secured excellent reduction of malaria rates.

In Panama and the Canal Zone, as well as through most of the lowlands of the Caribbean area including the West Indies, *Anopheles albimanus* is considered to be the principal vector of malaria. This species is wholly absent from dwellings during the daylight hours, or at best present in only small numbers. Pyrethrum sprays applied during the daylight hours in the manner of Russell, Knipe and Sitapathy would, therefore, probably meet with only indifferent success in the Caribbean area, although the recent work of Metcalf and Wilson (1945) might indicate that pyrethrins applied in heavy dosages on resting places would have residual effect. The realization of the special properties of DDT when applied

<sup>1</sup> The data and observations in this paper were obtained in connection with investigations conducted under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Gorgas Memorial Institute of Tropical and Preventive Medicine.

<sup>2</sup> 1st Lt., Sn.C., A.U.S.

to surfaces in the manner of a paint has aroused interest in the possibility of a new, simple and inexpensive approach to the control of malaria transmission—for DDT thus applied continues to kill and otherwise affect mosquitoes resting on treated surfaces for a prolonged period.

Even before the advent of DDT, interest in anti-adult spraying had been on the increase (Senior White and Rao, 1944, and Eddey, 1944). It has been superficially evident that the residual properties of DDT would be of particular value in treating dwellings. With the pressure for immediate results for the protection of Allied troops and civilian populations in malarious areas, vast numbers of dwellings were treated with DDT by the armed forces during the latter war years. The evaluation of the effectiveness of treatments of this sort is a difficult process at best. With the rapid movement of the war relatively little detailed information could be gathered.

A number of isolated experiments have been performed, however, and the results of this first work is now forthcoming in the publications of Senior White (1945), Gahan and Lindquist (1945), Gahan, Travis, Morton, and Lindquist (1945), Gahan and Payne (1945), Tarzwell and Stierli (1945), Knowles and Smith (1945), Metcalf, Hess, Smith, Jeffery and Ludwig (1945).

The work of Gahan and Payne (1945) on *Anopheles pseudopunctipennis* in Mexico is the only report of a residual spray experiment on a neotropical anopheline which has come to our attention at the time of writing, although we have visited other such experiments involving *Anopheles albimanus* in Veracruz, Mexico, and have word of work of this sort in progress elsewhere in tropical America.

#### THE EXPERIMENTAL AREA

In the summer of 1944, a field experiment was set up on the middle Chagres River, Panama, to study in detail the effectiveness of the DDT residual spray treatment of native dwellings in the control of malaria vectors and malaria. The area selected was one in which intensive studies of malaria and *Anopheles* have been carried on by Dr. H. C. Clark and his associates at the Gorgas Memorial Laboratory during the past fifteen years. The results of these various studies have been published over a period of years in this Journal, and in the Annual Reports of the Gorgas Memorial Laboratory.

The village of Gatuncillo was selected for treatment, while Guayabalito and Santa Rosa, adjacent villages, were used as controls. Concurrent observations were recorded for treated and control villages. All three of these settlements are located on the west bank of the Chagres River only a few feet above the water. The river here is ordinarily a sluggish stream with numerous backwaters and lagoons directly across from the villages being studied. (See plate I, figure 1.) Extensive dense mats of *Najas arguta*<sup>3</sup> provide the principal source of breeding for *Anopheles albimanus*, as well as protection from the numerous surface feeding minnows. In addition to the extensive *A. albimanus* breeding areas along the Chagres River proper, there is additional breeding of this species in small clear areas of tributaries of the Chagres, particularly behind the villages of Santa Rosa and Guayabalito.

<sup>3</sup> Determined by Prof. R. T. Clausen, Dept. of Botany, Cornell University.

In the past, water lettuce (*Pistia stratiotes*) was abundant along the Chagres River and supported breeding of *Anopheles triannulatus*. The lagoons and backwaters



PLATE I, FIG. 1

AERIAL PHOTOGRAPH OF THE MIDDLE CHAGRES RIVER AREA

The treated and control villages on the west bank of the Chagres River are shown as well as the location of the three stable traps in and near Gatuncillo. The extensive breeding area of *Anopheles albimanus* in the backwaters and lagoons of the east side of the river, directly across from the native villages, may be seen.

of this area were artificially created with the formation of Gatun Lake, and the succession of aquatic plants in them has been such that water lettuce is now a minor species and possibly in consequence *A. triannulatus* is now not commonly

encountered. Adult females of *A. albimanus* and *A. triannulatus* may only be differentiated in part. In the present study it has only occasionally been possible to hold female *Anopheles* until oviposition to secure positive identification, based on egg characters. No *A. triannulatus* type eggs were found among house caught mosquitoes. Eight years ago, Rozeboom (1938) found only 0.5 per cent of the house resting *Anopheles* in this area to be *A. triannulatus* (= *A. bachmanni*) although the species was breeding abundantly at that time, indicating that this species "does not care enough about human blood to enter the houses to feed on man at night". In the present study we therefore uniformly regard the *Anopheles* of the *Nyssorhynchus* group as *A. albimanus*.

*Anopheles punctimacula*, which is also present in the villages studied, breeds in small numbers in small shaded brooks which flow into the Chagres. The fluctuations in numbers of *A. albimanus* and *A. punctimacula* in dwellings and horse-baited stable traps in this area will be considered in a separate paper, but it may be stated here that throughout most of the year *A. albimanus* is dominant, with *A. punctimacula* being taken in significant numbers only at the end of the rainy season (i.e., October, November, and December).

The area in which this work is being conducted possesses several particular advantages for an experiment on the DDT residual spray control of malaria. These may be summarized as follows:

1. The area is one which is accessible to the investigator, and yet sufficiently isolated so that the native population moves relatively little. The villages studied are inaccessible except by dugout cayuco or foot trail. Local studies of malaria in populations located on roads tend to be confused by the uncertainty of determining just where infection was incurred since the natives can freely and conveniently move along the road to attend markets, fiestas, etc. In the present situation movements occur but they are less frequent. The Gorgas Memorial Laboratory is equipped with dugout cayucos provided with outboard motors enabling investigators to reach the Chagres River villages, some five and one half miles from Gamboa (the end of the road), in approximately forty-five minutes. Natives lacking motor driven boats require several hours of paddling for this trip and are thereby somewhat deterred from leaving the villages. Information derived from malaria rates is therefore relatively reliable although necessarily subject to interpretation in individual cases.

2. The writer has been singularly fortunate in being able to work at villages where the malaria in the populations has been under continuous study by Dr. H. C. Clark and his associates for the past fifteen years. Malaria rates based on thick film blood examinations are available on a monthly basis for ten years, and on a bimonthly basis for the last five years. We began our experiment therefore with a large backlog of information on malaria in these communities.

3. Conditions are excellent for the continuous production of large numbers of the malaria vector species, thus providing a most severe test of the effectiveness of the control method under study. The level of the Chagres River, in the study area, is maintained by the impounded Gatun Lake, and while there is some fluc-



tuation in water level, the breeding areas produce mosquitoes throughout the year. While some spraying with copper sulfate to kill aquatic vegetation is done by the Dredging Division of the Panama Canal, through agreement with the Chief Health Officer no larviciding procedures have thus far been employed in the study area. The confusing factor introduced by other means of mosquito control in the same area is largely eliminated and modifications in *Anopheles* abundance may be attributed either to natural causes, or to the techniques employed in the DDT residual spraying of dwellings.

4. The present study provides a severe and critical test of house spraying against adult female mosquitoes, since the species involved, *A. albimanus* and *A. punctimacula*, are not domestic as are, for example, *A. quadrimaculatus* or *A. gambiae*, which rest in human dwellings and domestic animal shelters during the day. While Rozeboom (1941) considers the adult female *A. albimanus* to be "very domestic" he also remarks that they "do not remain long in houses; most of them return to the jungle or to their breeding places soon after feeding or early in the morning".

Thus we are dealing with mosquitoes which seek dwellings not as resting places but as a source of a blood meal. If the residual house spraying method should prove successful against these species, it may be inferred that the technique will be even more effective against other malaria vector species which are house resting by preference.

5. The natives of the study area have been employed and trained as helpers and collectors by various members of the staff of the Gorgas Memorial Laboratory through the years and now constitute a good source of local assistants.

#### METEOROLOGICAL CONDITIONS

Meteorological information for the Chagres River villages is based on data from the Madden Dam Meteorological Station of the Panama Canal Section of Meteorology and Hydrography. This station is located approximately two and one half miles east of the village of Gatuncillo. Data for the period of the experiment reported here are given in table 1.

Weather conditions, particularly rainfall, are often very local in nature and the data from Madden Dam sometimes do not reflect conditions at the study villages. For example, in the severe storm and flood of December 13-15, 1944, which drastically affected the mosquito population, only 4.88 inches of rainfall were recorded at Madden Dam while 18.67 inches of rain fell at the Candelaria station about five miles away. In this same storm all twenty-four hour rainfall records for the Canal Zone area were broken, with 13.62 inches of rain recorded at Agua Clara. The annual rainfall at Madden Dam based on a forty-six year average is 98.25 inches.

The humidity at the Chagres river villages tends to be higher than that recorded at the Madden Dam Station since the latter is located on a hill above the river, while the villages are situated on the banks of the stream. On the occasions we have made hygro-thermograph records at Gatuncillo in the rainy sea-

son, we found the relative humidity to be virtually 100% throughout the night when the mosquitoes are active. Even in dry season the atmosphere becomes saturated by 10:00 or 11:00 p.m.

The data contained in table 1 will serve, however, to give a general picture of meteorological conditions in the region of the experimental villages.

#### NATIVE HOUSE CONSTRUCTION AND LIVING CONDITIONS

Construction in the villages of the middle Chagres River is of two sorts. The native huts are provided with steeply sloping palm-thatch roofs and walls of canes which are for the most part vertically arranged and secured with vines or, in some cases, wire (plate II, figures 2 and 4). Other houses are constructed of boards,

TABLE 1  
*Meteorological information, Madden Dam*

	PRECIPITATION (IN INCHES)		NUMBER OF RAINY DAYS	AVERAGE RELATIVE HUMIDITY (IN PER CENT) (8:00 A.M.)	TEMPERATURE (IN DEGREES FAHRENHEIT)		
	Actual	46 Year average			Maximum	Minimum	Mean
Oct. '44.....	22.92		28	93.4	90	69	78.9
Nov.....	10.66		18	91.5	89	66	78.6
Dec.....	6.63		17	87.5	88	68	78.4
Jan. '45.....	.20	.98	6	86.8	89	61	77.6
Feb.....	.02	.59	2	79.6	89	66	79.6
Mar.....	.04	.49	1	78.0	93	67	80.2
April.....	1.05	3.17	9	81.4	95	68	81.1
May.....	12.44	11.25	21	86.1	94	69	79.8
June.....	5.95	11.60	21	91.1	94	70	80.8
July.....	13.28	12.22	25	93.4	90	70	79.6
Aug.....	11.79	12.09	26	95.8	91	69	79.2
Sept.....	14.08	11.35	23	94.6	92	67	78.5
Oct.....	9.44	14.74	22	94.1	90	67	78.3
Nov.....	10.54	13.96	27	94.8	90	68	78.4
Dec.....	8.58	5.81	16	92.2	89	66	77.2

mostly salvaged from abandoned Canal Zone houses, and roofed with sheets of galvanized iron. Various of the houses are intermediate in construction, with thatch roofs and board walls (plate II, figure 3). For the most part, houses consist of a single room with an incomplete partition or two of cane or scrap lumber, and a small extension where cooking is done on an open wood fire. These extensions are usually roofed with several sheets of corrugated galvanized iron. Interior walls and partitions are either bare cane (plate II, figures 6 and 7), or "papered" with pages from magazines, or sheets of newspaper (plate III, figure 8). The natives sleep on rough board beds, or in hammocks slung across the main room. The canes comprising the walls and partitions are only loosely apposed to one another, providing numerous spaces through which mosquitoes can gain entrance and egress. Crude wooden shutters serve to close window openings at night. Glass and screens are nowhere in use.

Other than beds, furniture consists only of an occasional open shelf cupboard, and rough wooden tables, chairs, and benches. Extra clothes are hung from

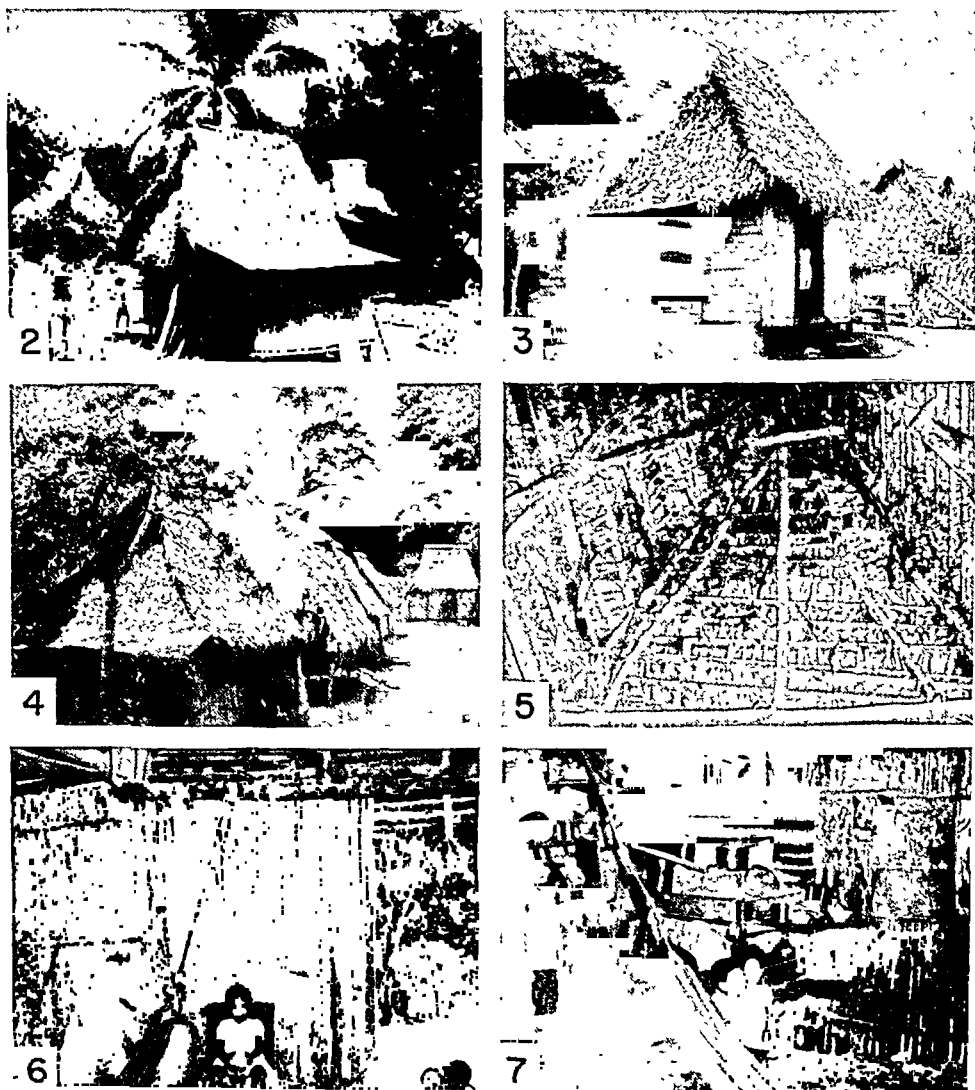


PLATE II

## NATIVE HOUSES AT GATUNCILLO

FIG. 2. Native house showing typical construction of cane walls and palm thatch roof.

FIG. 3. House showing intermediate type of construction with palm thatch roof and rough board walls.

FIG. 4. General view down "street" at Gatuncillo.

FIG. 5. Interior view of roof of palm thatch construction. The impossibility of calculating precise amounts of DDT deposited on surfaces like these is evident.

FIG. 6. Interior view of house with cane walls showing loose apposition of the canes permitting mosquitoes free access to the interior.

FIG. 7. Interior view of house showing crude cupboard and cooking arrangement.

wooden pegs or nails, or stored in chests. As decorations there are occasional framed pictures, or horns of deer. Paint is rarely used.

Houses constructed in the manner here outlined seldom last for more than a few years. Even during the fifteen-month period of the experiment reported here, there has been considerable tearing down of old houses and construction of new ones. The village of Gatuncillo is composed of about twenty-five houses while the control villages combined have about twice as many houses.

There is another type of native construction not employed in the villages used in this experiment, but frequent in the dryer portions of Panama and elsewhere in tropical America. These dwellings are about the same size, but the walls are plastered with mud, or mixtures of mud and dung. Others obtain a solid wall by constructing walls of adobe blocks, cement, or native stone. Construction of the latter types is, however, more common at some elevation (largely above the range of *A. albimanus*), where temperatures are lower and better shelter necessary.

TABLE 2

*Mosquito catches in houses at Santa Rosa and Guayabalito from October 1944 through December 1945*

	NUMBERS	PER CENT OF TOTAL CATCH
<i>Anopheles albimanus</i>	33,381	62.4
<i>Anopheles punctimacula</i>	1,009	1.9
Total <i>Anopheles</i>	34,390	64.3
<i>Mansonia titillans</i>	16,225	30.3
<i>Mansonia nigricans</i>	2,605	4.9
Other Culicines	292	.5
Total Culicines	19,122	35.7
Total, all species	53,512	100.0

#### THE MOSQUITO FAUNA

Mosquitoes taken in the houses are principally of four species; *Anopheles albimanus*, *A. punctimacula*, *Mansonia titillans*, and *M. nigricans*. These mosquitoes are almost wholly absent from the houses during daylight hours, appearing at dusk, and departing at or shortly after dawn. Houses in which as many as 500 or 1000 mosquitoes may be taken by collecting throughout the night will have fewer than a dozen, or even no mosquitoes at all during the daylight hours.

A summary of mosquito catches in houses in the untreated villages of Santa Rosa and Guayabalito for the fifteen-month period from October 1944 to December 1945 is given in table 2.

An examination of table 2 shows that of 53,512 house-resting mosquitoes taken throughout the year almost two-thirds were *Anopheles*, mostly *A. albimanus*.

#### ENTOMOLOGICAL METHODS

While the malaria rate is the ultimate criterion of the effectiveness of the DDT house spraying method, some entomological check on the villages was obviously

desirable, not only to keep a close record on how the vectors were being affected, but also to provide a basis for decision on the time of retreatment. At first the attempt was made to secure evening biting rates on native human subjects, but these were soon found to be too variable to be of value, since the time of flight of the anophelines varies considerably with local meteorological conditions. This technique was therefore soon abandoned. Horse-baited stable traps were found to give a more reliable index of outdoor mosquito activity. The traps used were of the Magoon type with the ingress baffles modified as in the Egyptian trap of Bates (1944). Horses were purchased so that throughout the course of the experiment the same horse might be used in the same trap, thus avoiding any error caused by differences in the attractiveness of individual animals pointed out by Bates (1944).

To avoid the curious "catching-out" phenomenon reported by Gabaldon, Lopez, and Ocho-Palacios (1940), traps were never operated on successive nights. A schedule of trapping on Monday and Thursday nights of each week was set up and has been adhered to throughout the course of the experiment. Furthermore, at no time have the traps been moved from their original locations. By these precautions we hope to have established a high degree of reliability in the horse trap catches, permitting valid comparisons of catches over an extended period.

At first, three traps were set up: one across the Gatuncillo River about three hundred feet north-east of the village of Gatuncillo, a second in the center of the village, and a third in the forest on the bank of the Chagres River about nine hundred feet south of the village. These traps commenced operation on October 10, 1944. Later three additional traps of the same sort were set up. One in the village of Guayabalito 1.3 miles south of Gatuncillo, a second about 0.4 miles west of Guayabalito, behind a low ridge along the Chagres River, and a third at Juan Mina, 3.2 miles south of Gatuncillo on the east bank of the Chagres River. The locations of the first three traps are indicated in plate I, figure 1. Mosquitoes in stable traps were collected with a suction tube and chloroformed for subsequent identification and counting in the laboratory.

Several methods of collecting mosquitoes in houses were used before a satisfactory technique was evolved. At first chloroform tubes were used. These proved satisfactory for catching resting mosquitoes, but since DDT has a delayed action it was obviously desirable to collect the mosquitoes alive and hold them under favorable conditions to observe subsequent mortality. Catching tubes consisting of ten mm. glass tubing, in one foot lengths, with bolting cloth covering one end, and an attached rubber tube about two feet in length proved satisfactory for this purpose. As holding cages for the mosquitoes, we at first used glass lamp chimneys, with their ends covered with bolting cloth. If these were kept in a dry atmosphere the mosquitoes died; if kept in a moist atmosphere, slight changes in temperature would produce a condensation of moisture on the walls of the chimneys. On alighting, mosquitoes would adhere to the walls and perish. It was suggested by Col. W. H. W. Komp that holding cages of wire mesh or a similar material would overcome this difficulty. Cages of wire mesh were too fragile in the hands of native collectors, and for transportation between the field and laboratory. A series of cages were therefore constructed of per-

forated sheet bronze, four and a half inches in diameter and six inches tall. These were provided with a number eighteen wire screen bottom for convenience in examining the contents of the cage, and a bronze removable cover with a hole three-fourths of an inch in diameter. Through the hole in the cover mosquitoes could be introduced, by blowing, from the catching tubes. The removable feature of the cover was a convenience in cleaning the cages.

For transporting the cages of mosquitoes from the river villages to the laboratory we had constructed carrying boxes 9" x 11" x 22". These boxes contained eight compartments in the bottom of each of which a petri dish with moist cotton was placed. The holding cages were thus carried in separate compartments, each over moist cotton, and in an atmosphere of saturated humidity. All catches of house resting mosquitoes were handled in the same fashion, the mortality among the mosquitoes from the untreated villages serving as a control for that in the mosquitoes from the treated village. Catches were held in these cages for twenty-four hours, at which time the dead mosquitoes were removed, identified and counted. The surviving mosquitoes were then chloroformed and examined.

The catches of mosquitoes from houses were not only identified and counted, but also sorted as to whether or not they were blood engorged. Cards (5" x 8") were printed on which the information for each house, for each night's catch, was recorded. From these cards it is possible to tell at a glance the numbers of mosquitoes of each species which are engorged or unengorged, and the twenty-four hour survival. Summary cards for each house and each village were also routinely prepared at the end of each month.

House catches made during the daylight hours would in no way reflect the nature of the anopheline population visiting these dwellings. We therefore established a routine of collecting in houses after dusk, during the evenings, and mornings before dawn. Native boys were employed who collected in the house in which they were resident. It was not possible to establish an exact time period during which the boys collected, but each was instructed to catch all the mosquitoes resting in his dwelling between dusk and bedtime. In practice this was the period from about 6:30 p.m. to 9:00 p.m. In the morning the boys collected approximately between the hours of 5:30 and 6:30 a.m. No premium was placed on the numbers of mosquitoes collected, boys receiving a fixed sum for each night's collection no matter how large or small. Thus was avoided the temptation of the natives to secure mosquitoes from other sources than their own houses. On the whole this method has worked out satisfactorily. One native who persisted in collecting mosquitoes off tethered animals, when his house catches were low, was discharged.

While the interior of dwellings are exceedingly irregular and resting mosquitoes are in consequence not easy to see, the native boys quickly become adept collectors and a comparison of the catches from different houses on the same night shows a good correlation.

#### DDT VILLAGE TREATMENT

While it was at first thought necessary to spray a belt of vegetation around the village to be treated, a preliminary study of the habits of *A. albimanus* in-

licated that spraying of the houses alone might be sufficient. A comparison of catches of mosquitoes taken in dwellings in the evening with those taken just before dawn showed a higher percentage of engorgement in the morning-caught mosquitoes. It was thus indicated that mosquitoes which have fed tend to rest on the conveniently nearby house surfaces for a period, before flying off to a more agreeable microclimate elsewhere for the daylight hours. This behavior characteristic of *A. albimanus* is of critical importance in the consideration of the effectiveness of the DDT house spraying technique in the control of malaria transmission. The "seed bed" of malaria is in the village dwellers and the engorged mosquitoes resting in the dwellings are, in consequence, those potentially infected. By selectively killing those mosquitoes which have fed on the occupants of the houses we may hope to control or terminate malaria transmission without necessarily exterminating, or even seriously modifying the mosquito population of an area.

It was early appreciated that the line of reasoning upon which the success of this method of using DDT rests is dependent on two basic premises. First, it was considered necessary that the human population be a relatively domestic one; i.e., a population which is for the most part indoors during the period of anopheline activity. The natives of the area here studied do not wholly meet this requirement. In the evening they are often abroad in the village, particularly during fiestas. The men are sometimes absent from the village when they remain overnight in shelters on the hillsides some distance from the village where they cultivate upland rice and corn. Data which will be presented below, however, tend to show that this difficulty is not insurmountable. The second premise is that the blood-engorged mosquitoes found in the dwellings are actually those which fed on humans and not mosquitoes which have fed elsewhere on animals other than humans and merely sought shelter in the dwellings. We have been able to check this point by precipitin test analysis of the blood-meals of house-resting mosquitoes.

Preliminary studies of the relation of *A. albimanus* to native dwellings, of the sort described above, showed that in large measure the mosquitoes approaching dwellings rest on the outer wall of the houses, and walk between the canes to the interior. Frequently, too, they enter by the space between the thatch roof and the walls. When houses of cane and thatch were provided with window traps it was found that approximately ninety per cent of the mosquitoes in a dwelling entered by some means other than the doors or windows. Further evidence that most mosquitoes find ingress to the dwellings by some means other than the doors and windows is found in the fact that at night the natives close the doors and shutters to their houses. Nevertheless hundreds of mosquitoes may be collected in these houses at this time.

It was therefore decided to spray the exteriors as well as the interiors of all dwellings so that the opportunity for mosquitoes to contact DDT might be enhanced. Inside the dwellings not only walls, but also any other possible resting places of mosquitoes, as the undersides of chairs, tables, cupboards, beds, and false ceilings were also sprayed. At the time of the first spraying, equipment was not available to reach the underside of the tall peaked roofs, but in subsequent

treatments these surfaces were also sprayed. Outside the houses the undersides of the overhanging eaves were sprayed as well as the walls. In addition to the human habitations all outhouses and animal shelters, such as chicken coops, were also sprayed inside and out.

A statement of the precise rate of application of DDT in terms of milligrams per square foot would be desirable, but is not feasible in view of the type of house sprayed in this experiment. While the plane surface area of walls, partitions, and roofs may be easily calculated, the rounded surface of the canes of which most walls and partitions are constructed (plate II, figure 6), and the multiple surfaces presented by the undersides of the palm thatch roofs (plate II, figure 5) and eaves makes it virtually impossible to establish the actual surface area treated with DDT. A more useful statement of the rate of application of DDT is the mean volume of DDT solution applied per house. Treating houses as outlined above, including the inner and outer walls, interior partitions, false ceilings, undersides of peaked roofs, eaves, and the undersides of furniture, we find that we use approximately three gallons of solution per house. A relatively uniform rate of application of the DDT solution was obtained by training spray operators to wet surfaces without permitting the solution to run. Close supervision of the work is necessary to secure uniform results. Since the construction of these houses is similar, both in terms of construction materials and size, throughout the Caribbean lowlands, this figure for the amount of material used should have wide application in this area.

Where house construction is more substantial, with the cane walls plastered with mud, or with walls of adobe, stone, or cement, the amount of DDT solution necessary per house is materially reduced since spraying inside alone should suffice, for mosquitoes do not gain entrance through the walls as in the circumstances of the present experiment. The relative effectiveness of DDT on surfaces of this sort is yet to be determined in the field.

#### EQUIPMENT FOR VILLAGE SPRAYING

The material used for the spraying was five per cent technical grade DDT in kerosene which was prepared from Army stock DDT and locally obtained kerosene. Various methods of applying this solution were tried. The work was mostly done with a small portable air-compressor such as is used with the paint-spray equipment for camouflaging jungle positions. This consists of a three-quarter horse-power air-cooled gasoline engine which operates a small compressor capable of delivering pressure of sixty pounds per square inch. This unit is easily carried by two men. An air line runs from the compressor to a liquid pressure tank of five gallons capacity. Attached to this tank are two hoses, one carrying liquid, the other air. These are then attached to a pistol-type spray gun which delivers a fan spray which is excellent for the purpose of applying an even coat of DDT solution. (See plate III, figures 9 and 10.) In practice, however, this equipment was found to be not wholly satisfactory. The two hoses from the liquid tank to the sprayer were cumbersome and permitted too restricted an area of movement by the spray operator, since the hoses were but thirty feet long.



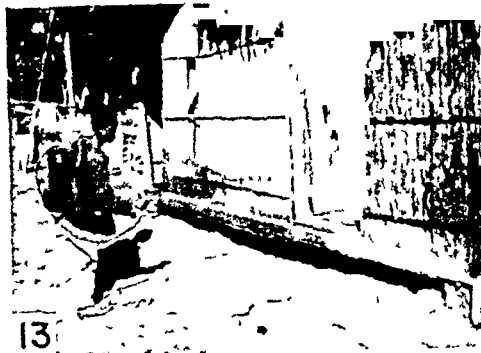


PLATE III

### NATIVE HOUSES AND METHODS OF APPLYING DDT

FIG. 8. Rough board partition "papered" with magazine covers and pages.

FIG. 9. Apparatus used in first spraying of Gatuncillo. In the foreground is the portable unit consisting of a  $\frac{3}{4}$  horsepower air-cooled motor and air compressor. The five gallon tank containing the DDT solution is beside the figure at the left, the operator at the rear.

FIG. 10. The pistol-type spray gun used with this apparatus is shown here. Two hoses from the insecticide tank may be seen; that at the right delivers the insecticide, that at the left compressed air. A wet fan-type spray is used.

FIG. 11. For the second spraying a modified technique was used. A single hose from the insecticide tank is provided with the nozzle from a knapsack sprayer. This is a less cumbersome arrangement.

FIG. 12. In a later spraying hand-pumped cylindrical knapsack sprayers were used. The fan-type spray can be seen.

FIG. 13. The systematic coverage is accomplished by having the operator move slowly back and forth along the wall holding the spray nozzle at a uniform distance from the surface. A wet treated surface can be seen at the right and on the lower part of the wall section which the operator is spraying.

For a subsequent treatment the equipment was modified by substituting for the pistol-type spray-gun requiring two hoses, the disk-type spray nozzles removed from knapsack sprayers. These were provided with a number sixty wire gauge aperture which gave a wet spray satisfactory for this work. These sprayers required only a single hose, which simplified the work for the operator. (See plate III, figure 11.) To speed up the spraying, two and even three liquid lines could be run from the same tank, since only ten or fifteen pounds of pressure was necessary on each line to secure a satisfactory spray, and the compressor was capable of delivering as much as sixty pounds pressure per square inch. With this type of sprayer, hose extensions could be added so that each operator might have one hundred or one hundred and fifty feet of hose, thus allowing him great freedom of movement.

The fault with this arrangement lay in that a five gallon liquid tank contained sufficient liquid for only about fifteen minutes spraying and too frequent interruptions were necessary to refill the tank. We later improvised a liquid tank from a grease barrel which would hold fifteen gallons of liquid and this proved satisfactory.

Nevertheless it was considered desirable to try the village spraying with manually pumped cylindrical sprayers since labor costs are relatively low and the difficulty of effecting repairs to mechanical equipment in remote places is great. The simplest sort of spray equipment which will perform the work required is the most desirable under the conditions encountered in this area and Central America generally. In a field trial it was found that manually pumped cylindrical sprayers, of three or four gallons capacity, provided with a nozzle delivering a fan spray were excellent. The sprayers used were regular items of Army issue. (See plate III, figures 12 and 13.)

In the present experiment no attempt was made to develop ideal equipment since facilities for this sort of work were limited, and the village involved was not large. With the equipment here outlined we were able to treat the entire village in two days using two spray operators and an assistant.

Operators were supplied with respirators and heavy rubber gauntlet gloves, but these were frequently discarded since they were too uncomfortable for operators working in this tropical climate. Nevertheless, no untoward effects were observed in operators with the exception that one person using a spray gun which leaked as he extended his arm to spray high portions of walls sustained a kerosene burn on his arm and right side of his torso. This cleared up in a few days with no sequelae.

A more extended account of equipment for work of this sort may be found in the paper by Stierli, Simmons, and Tarzwell (1945).

#### ENTOMOLOGICAL RESULTS

##### *House Catches*

*Mosquito abundance.* The fluctuations in house catches of *Anopheles* and the principal factors influencing them are shown in Chart I and table 3. While the

catches were made semi-weekly (each Monday and Thursday night), in this and succeeding charts and tables the average for each week is shown to simplify presentation of the large volume of data available. Points of interest may be enumerated as follows:

1. In the pretreatment month, October 1944, the numbers of *Anopheles* in both the village to be treated and the control villages showed weekly fluctuations, but

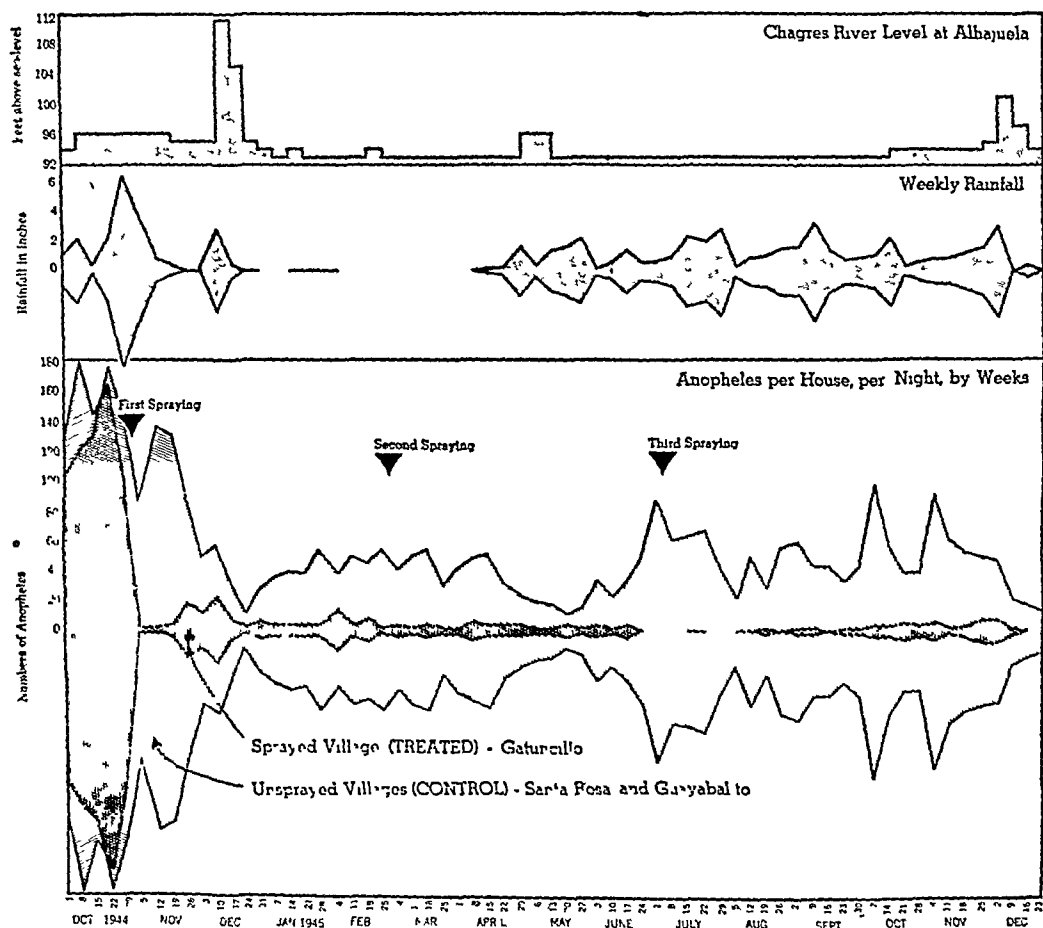


CHART I. CHART OF ANOPHELINE ABUNDANCE IN HOUSES OF TREATED AND CONTROL VILLAGES CORRELATED WITH RAINFALL AND RIVER LEVELS, MIDDLE CHAGRES RIVER, PANAMA

maintained a high level of abundance (from 99 to 178 *Anopheles* per house, per night). At this time the rainfall was relatively high.

2. With the first spraying of Gatuncillo, which took from the 31st of October to the 2nd of November, there was a dramatic drop in the numbers of *Anopheles* in the treated village, the average number of *Anopheles* per house, per night, during the ensuing three weeks being from 1.8 to 4.5. A gradual rise during the succeeding three weeks to 21.5 in the week of the 10th of December followed. During this six week period there is a decline in the numbers of *Anopheles* in the control villages associated with the drop in rainfall and the beginning of dry

TABLE 3

*Anopheline abundance in houses of treated and control villages correlated with rainfall and river levels*

WEEK OF	AVERAGE NUMBER OF ANOPHELES PER NIGHT		PRECIP. AT MADDEN DAM (IN INCHES)	MAX. RIVER LEVEL (IN FEET)	WEEK OF	AVERAGE NUMBER OF ANOPHELES PER NIGHT		PRECIP. AT MADDEN DAM (IN INCHES)	MAX. RIVER LEVEL (IN FEET)
	Control	Treated				Control	Treated		
Oct. 1, '44	102.3	128.5	2.12	94	May 6	19.5	1.5	.71	96
8	119.7	178.5	4.14	96	13	17.7	2.0	2.54	93
15	129.0	144.3	.68	96	20	10.9	3.3	3.20	93
22	174.9	163.0	4.37	96	27	15.2	1.3	4.24	93
29	142.2	99.0	12.55	96	June 3	33.0	3.5	.33	93
First DDT Treatment					10	23.2	1.3	.99	93
Nov. 5	87.3	1.8	7.51	96	17	34.6	4.0	2.69	93
12	135.2	2.3	1.55	96	24	50.3	0.5	1.27	93
19	130.3	4.5	.56	95	Third DDT Treatment				
26	85.9	18.0	.12	95	July 1	87.8	0.0	1.44	93
Dec. 3	49.9	12.3	.06	95	8	62.5	0.0	1.95	93
10	55.3	21.5	5.57	111	15	63.6	0.8	4.52	93
17	29.9	5.8	.96	105	22	67.5	0.3	4.07	93
24	11.2	1.3	.02	95	29	42.4	0.0	5.58	93
31	27.5	6.5	.03	94	Aug. 5	23.1	0.3	.80	93
Jan. 7, '45	35.9	4.3	.00	93	12	50.2	0.5	1.83	93
14	39.0	3.3	.01	94	19	29.4	1.5	1.96	93
21	37.9	4.3	.15	93	26	55.0	0.8	3.06	93
28	53.1	4.5	.02	93	Sept. 2	60.5	2.8	3.21	93
Feb. 4	37.5	13.5	.01	93	9	44.8	2.5	6.36	93
11	49.5	4.0	.00	93	16	43.8	0.5	2.79	93
18	45.0	7.3	.00	94	23	34.8	1.3	1.50	93
25	54.4	2.0	.00	93	30	43.4	1.8	1.87	93
Second DDT Treatment					Oct. 7	98.7	3.8	2.19	93
Mar. 4	40.4	3.5	.00	93	14	56.7	4.8	4.33	93
11	49.2	2.3	.00	93	21	60.5	6.3	.62	94
18	53.9	3.0	.04	93	28	59.6	4.0	1.19	94
25	30.6	1.3	.00	93	Nov. 4	91.9	5.0	1.66	94
April 1	42.4	1.0	.00	93	11	62.8	1.3	3.66	94
8	47.3	6.8	.09	93	18	53.7	3.5	2.39	94
15	52.3	4.0	.46	93	25	49.3	7.3	3.16	95
22	32.1	4.5	.47	93	Dec. 2	47.0	7.0	6.02	103
29	23.7	3.8	3.14	96	9	21.9	1.0	.02	97
					16	17.4	0.8	.85	94
					23	13.2	3.0	.11	93

season conditions. The week of the 10th of December the anopheline abundance in the control villages had about reached the usual dry season level. (Compare the portion of the chart for the dry season months of February and March.)

3. The week of the 10th of December was marked by a heavy rain, widespread over the entire Chagres River Basin, such as often occurs at the end of the wet

season. In this instance, however, the rain was of unusual intensity and duration. The recorded rainfall of 5.57 inches for this week at Madden Dam gives only a slight indication of conditions. Madden Lake rose so rapidly that it became necessary to discharge water from Madden Dam, producing a sixteen foot rise in the Chagres River at Alhajuela (see Chart I). The addition of a heavy inflow of water from streams tributary to the Chagres River below Alhajuela effected a rise of even more than sixteen feet at Gatuncillo.

This flood swept throughout the lowlands along the Chagres, carrying with it great masses of vegetation and even floating islands supporting trees. The decline in the control anopheline population during the latter half of December may reasonably be explained as the consequence of the destruction of so many mosquitoes by the torrential rains, and the sweeping out of daytime resting places as well as breeding mats of vegetation. The decline in numbers of *Anopheles* at Gatuncillo at this time is probably also attributable to this flood and is not a DDT effect.

By the end of January, however, the population at the control villages had been restored and reached a level maintained during the remainder of the dry season. The treated village, on the other hand, showed no such rapid recovery, the numbers of *Anopheles* per house, per night, seldom rising above five. These data imply that following the flood the DDT on surfaces in the treated village was able to prevent recovery of the anophelines to their normal dry season level as established in the control villages.

4. The decision to respray Gatuncillo at the beginning of March, four months after the original spraying, was not influenced by any increase in numbers of mosquitoes, but by the fact that the twenty-four hour survival rate of blood-engorged *Anopheles* was no longer reduced. (This point will be discussed later in this paper.)

5. The decline of the anopheline population in the control villages following the week of the 15th of April is associated with the rains of the beginning of the wet season, and a subsequent three foot rise in the river level at Alhajuela. This decline may be due to a flooding of breeding beds reducing anopheline production. In any event, the low level of anopheline abundance in the treated village is unaffected.

6. Following the third spraying of Gatuncillo at the beginning of July, virtually no *Anopheles* were taken in the house catches at this village despite the fact that this was a time when *Anopheles* were on the increase in the control villages. It will be noted that following each spraying there are progressively fewer mosquitoes in the houses of the treated village. This may well be due to a cumulative effect of the DDT, when the period for respraying is as short as four months. It might be anticipated, therefore, that routine DDT residual sprayings of villages at four-monthly intervals will progressively, over a period of years, produce better and better control of *Anopheles*.

7. With the excellent control effected after the third spraying of Gatuncillo it was decided to permit a six month interval to elapse before the fourth retreatment, to determine whether this interval rather than four months might not be

satisfactory (reducing costs in labor and material by one third for the year). Examination of the chart for the latter part of this period (October, November, and December, 1945) shows a gradual increase of *Anopheles* in the treated village. From these data it appears advisable to retain the four-month period for retreatment, by way of insuring complete control.

8. At the end of the 1945 wet season (October and November) the house collections of *Anopheles* in the control villages did not reach the levels of the same period the previous year. There are unfortunately no data available for com-

TABLE 4

Summary showing per cent of mosquitoes engorged, based on semi-weekly catches totalling 43,878 specimens

	ANOPHELES		CULICINES		ALL MOSQUITOES		
	Control	Treated	Control	Treated	Control	Treated	
Nov. '44	74.3	4.3	57.4	8.7	73.3	5.8	Period following first treatment
Dec.	51.9	3.7	33.6	7.5	46.3	5.6	
Jan. '45	45.0	16.1	33.8	13.1	39.4	13.7	
Feb.	46.7	7.3	45.8	17.9	46.3	13.2	
Mar.	46.8	4.6	32.7	12.1	41.0	7.7	Period following second treatment
April	45.4	11.1	36.3	0.0	40.9	6.3	
May	52.3	12.8	44.0	17.8	48.2	16.0	
June	55.1	2.7	36.9	8.3	47.6	5.5	
July	46.8	0.0	46.1	4.3	46.6	3.7	Period following third treatment
Aug.	42.6	16.7	32.1	5.6	38.8	8.3	
Sept.	31.9	3.6	27.5	8.7	29.9	6.8	
Oct.	27.4	6.8	27.4	3.8	27.4	6.0	
Nov.	31.4	17.6	35.5	10.2	33.3	13.2	
Dec.	48.7	43.9	34.0	46.9	39.9	45.4	
<sup>1</sup> Average Nov. '44-Oct. '45	47.4	7.1	35.4	11.1	43.1	9.4	

<sup>1</sup> These percentages are based on the totals of mosquitoes taken in houses from November 1944 to October 1945. November and December 1945 are excluded since they represent the fifth and sixth months respectively after a treatment period.

parison from years prior to 1944, but annual variations of the magnitude shown in this chart may not be wholly unexpected. It is also possible that native collectors were somewhat less diligent in their work after more than a year of routine collecting, and did not expend so great an effort in house collecting as they did the year previous.

*Mosquito engorgement.* The reduction in numbers of *Anopheles* in the houses of the treated village, as demonstrated above, indicates a large measure of protection to the village residents. The reduction in numbers of *Anopheles* represents, however, only the first step in the direction of terminating malaria transmission by DDT residual house spraying. In December 1944, several weeks

after the initial village spraying we made the following observation (Trapido, 1944):

"It is also noteworthy that of 135 mosquitoes collected in dwellings since the spraying of the village only four, or 2.9%, have contained blood. It is indicated that mosquitoes entering the treated dwellings and contacting the DDT residue become so activated that they do not feed."

We now have the records on the per cent engorgement in house-caught mosquitoes for fourteen months. These are summarized by months in table 4.

The marked and consistent difference between the per cent engorged in the control and treated villages is at once apparent. If we exclude those periods more than four months after a DDT spraying we find that house-caught *Anopheles* are from 27.4% to 74.3% engorged in control villages, while they are from 0.0% to 16.7% engorged in the treated village. Only in December 1945 did the per cent engorgement in mosquitoes from the treated village approximate that at the control villages. This was the sixth month after treatment, and it seems evident that after so long a period as this the "activating" effect of DDT does not persist. When retreatments with DDT occur at four-month intervals, the per cent engorgement of such *Anopheles* as do enter the dwellings remains at a low level.

If we summarize the percentages for the year November 1944 to October 1945, including three four-month post-treatment periods, we find that of *Anopheles* 47.4% were engorged in the control villages while only 7.1% were engorged in the treated village. In table 4 are also summarized the data for culicine mosquitoes. These data conform in a general way to the results obtained with the anophelines.

Just how the DDT spraying of dwellings serves to reduce the percentage of engorged mosquitoes is a point of much interest. As has been noted earlier in this paper, field observations have shown that most *A. albimanus* alight on the outer surface of the native dwellings and then enter between the canes of the walls or by other crevices, either by walking or by short flights. Once in the dwelling they rest on the walls, ceiling, or furniture, waiting a favorable opportunity to feed. Those mosquitoes not sufficiently affected by the DDT on these surfaces to die, or fly off to die subsequently, may receive sufficient DDT to cause them to lose interest in feeding. This "activating" or "irritating" effect produces an overall reduction in feeding. Effects of this sort are confirmed by the recent observations of Metcalf *et al.* (1945) on *A. quadrimaculatus* in the Tennessee Valley. They made observations in a DDT-treated house and a control house nearby and observed, "Although many mosquitoes entered both buildings, only 4 bites were received by the person in the control house." While there was some increase in the biting rate in the treated house after eleven days, they note that, "An estimated 500 mosquitoes entered the building during a 15-minute period at the break of dawn (5 a.m. to 5:15 a.m.), but only a few of them took blood meals".

This reduction of feeding on the part of such *Anopheles* as may be found in

treated dwellings, due to the "activating" effect of DDT, may be considered the second step in the possible reduction of malaria transmission.

*Mosquito survival.* There is yet a third way in which the house spraying with DDT serves to reduce malaria transmission. As has been mentioned above we have evidence that blood engorged mosquitoes tend to rest on house surfaces for a period before flying off to seek a diurnal resting place. It is of interest, there-

TABLE 5

*Per cent of mosquitoes which are not engorged and survived 24 hours, based on semi-weekly catches totalling 38,538 specimens*

	ANOPHELES		CULICINES		ALL MOSQUITOES		
	Control	Treated	Control	Treated	Control	Treated	
<sup>1</sup> Nov. '44							Period following first treatment
<sup>1</sup> Dec.							
Jan. '45	21.1	21.0	32.0	32.4	26.5	30.0	
Feb.	27.0	43.1	32.7	53.7	29.4	49.0	
Mar.	42.1	44.6	58.3	72.7	43.8	54.1	Period following second treatment
April	46.8	61.9	58.1	83.7	52.5	71.4	
May	42.5	59.6	51.5	58.3	46.9	58.8	
June	33.2	89.9	52.4	88.9	41.1	89.0	
July	33.8	0.0	36.5	47.8	34.3	40.7	Period following third treatment
Aug.	30.3	33.3	35.9	75.0	32.3	64.6	
Sept.	34.2	21.4	38.3	36.9	36.0	31.1	
Oct.	48.8	18.9	39.8	38.5	45.9	24.0	
Nov.	37.9	31.1	34.6	46.3	36.6	40.1	
Dec.	25.1	31.7	29.6	35.7	27.8	34.0	
<sup>2</sup> Average Jan. '45-Oct. '45	36.7	41.7	44.0	49.5	39.6	46.4	

<sup>1</sup> Data on survival rates not available for these months.

<sup>2</sup> These average percentages are based on the totals of mosquitoes taken in houses from January to October 1945. November and December 1945 are excluded since they represent the fifth and sixth months respectively after a treatment.

fore, to examine the survival rate in engorged and unengorged mosquitoes taken in dwellings, and to compare these rates in treated and control villages.

Data on survival of mosquitoes from both control and treated houses were routinely recorded from January 1945 onward. It was not until then that a standardized procedure for handling living mosquitoes and recording data on survival rates was established. We have recorded, however, (Trapido, 1944) that it was not until the 26th day after the first treatment that even a single mosquito from the treated houses survived for twenty-four hours after capture.

We have already outlined under "Entomological Methods" the technique employed to hold mosquitoes under favorable conditions to observe the twenty-four hour survival rate. These observations are summarized by months in



tables 5 and 6. Table 5 shows the per cent of all mosquitoes which were both unengorged and survived twenty-four hours. It is to be noted that, on the whole, for both *Anopheles* and culicines, there is little difference in the percentages recorded from the control and treated houses, although there are considerable monthly fluctuations. The wide range of percentages recorded for *Anopheles* in the "treated" column is due to the fact that these percentages are derived from relatively small numbers of specimens (since the DDT treatment enormously

TABLE 6

*Per cent of mosquitoes which are engorged and survived 24 hours, based on semi-weekly catches totalling 88,538 specimens*

	ANOPHELES		CULICINES		ALL MOSQUITOES		
	Control	Treated	Control	Treated	Control	Treated	
<sup>1</sup> Nov. '44							Period following first treatment
<sup>1</sup> Dec.							
Jan. '45	40.0	1.2	28.1	3.9	33.6	3.4	
Feb.	39.9	7.3	38.8	10.4	39.4	9.1	Period following second treatment
Mar.	42.9	3.1	31.1	12.1	38.0	6.1	
April	41.5	7.9	35.8	0.0	38.6	4.5	
May	50.1	8.5	43.3	16.7	46.8	13.7	
June	52.4	2.7	35.7	8.3	45.5	5.5	
July	41.2	0.0	38.3	0.0	40.7	0.0	Period following third treatment
Aug.	37.5	0.0	24.9	2.8	33.0	2.1	
Sept.	29.1	3.6	23.0	4.3	26.4	4.1	
Oct.	25.2	0.0	23.2	3.8	24.6	1.0	
Nov.	26.8	10.8	30.9	10.2	28.4	10.4	
Dec.	42.3	43.9	28.5	42.9	34.1	43.3	
<sup>2</sup> Average Jan. '45-Oct. '45	38.3	4.2	31.3	6.6	35.5	5.7	

<sup>1</sup> Data on survival rates not available for these months.

<sup>2</sup> These average percentages are based on the totals of mosquitoes taken in houses from January to October 1945. November and December 1945 are excluded since they represent the fifth and sixth months respectively after a treatment.

reduced the overall population). For the period from January to October 1945 there was actually a greater percentage of unengorged mosquitoes surviving twenty-four hours from the treated houses, than from the control houses, although this is probably a chance rather than a significant difference.

The data for the engorged mosquitoes which survived twenty-four hours are quite different (table 6). These data are of particular significance for malaria transmission, since they concern the potentially infected mosquitoes. The following points are noteworthy:

1. The twenty-four hour survival rate in mosquitoes from the control houses was relatively high. Reference to tables 4 and 6 shows that of *Anopheles col-*

lected in the control houses in the four-month post-treatment periods 47.4% were engorged, and 38.3% were both engorged and survived 24 hours. Thus among engorged *Anopheles* taken in untreated houses there was approximately 80% survival; such mortality as occurred being due to a combination of natural causes and the imperfections in our methods of transporting and holding these mosquitoes.

2. The reduced percentages of mosquitoes from the treated houses which were both engorged and survived twenty-four hours are striking. Thus for the period January to October 1945 only 4.2% of anophelines from treated houses were engorged and survived twenty-four hours as compared with 38.3% from the control houses. The comparison of percentages for culicines is approximately the same.

3. A comparison of tables 5 and 6 will also show that even in the treated houses the percentage of *engorged* anophelines surviving twenty-four hours (4.2%) is markedly lower than for *unengorged* anophelines surviving twenty-four hours (41.7%). The fact that engorged *Anopheles* show a decidedly greater mortality than unengorged individuals provides indirect evidence that the engorged *Anopheles* have a longer contact period with DDT. This is a consequence of the behavior characteristic we have already noted, i.e., engorged mosquitoes tend to rest for a period on convenient nearby surfaces before flying off with their heavy blood meal. In effect there is a selective killing of the engorged mosquitoes, a matter of primary significance in understanding the reduced malaria transmission potential.

4. It is of special interest to note the percentages in both tables 5 and 6 for December 1945, the sixth month after the third spraying. We find that DDT effects on engorgement and survival seem wholly wanting, there being no significant differences between control and treated houses. It is thus indicated that six months is too long an interval between retreatments.

5. We have earlier mentioned that the criterion for retreatment at the end of the first four-month period (March 1945) was not an increase in total numbers of anophelines, but the lack of reduction in twenty-four hour survival rate. If we compare, for February and June 1945, the percentages of *Anopheles* from the treated dwellings which are engorged (table 4) with those which are both engorged and survive twenty-four hours (table 6) we find that they are the same. It is thus demonstrated that while in the fourth month after treatment the blood-engorgement is still reduced, there is no twenty-four hour mortality among these engorged *Anopheles*. The lack of "delayed" mortality in the fourth post-treatment month might be taken as the criterion for retreatment at the end of three months. The total reduction of *Anopheles* and the low rate of engorgement in those which are found during the fourth month seem, however, to combine to give adequate protection despite the lack of "delayed" mortality. Beyond this point retreatment is indicated.

*Summary.* It is now of interest to review the possibilities of malaria transmission in the light of the "three step" control of vectors demonstrated above.

A summary of the progressive effect of each of the three steps is contained in table 7.

The per cent reduction of blood-engorged *Anopheles* for each four-month post-treatment period will be found to be even greater than that recorded under "All *Anopheles*". The blood-engorged *Anopheles* surviving twenty-four hours

TABLE 7

Average number of *Anopheles* per house, per month, based on semi-weekly catches totalling 37,406 specimens

	ALL ANOPHELES				BLOOD-ENGORGED ANOPHELES				BLOOD-ENGORGED ANOPHELES SURVIVING 24 HOURS			
	Control	Treated	Ratio of treated to control	Per cent reduction	Control	Treated	Ratio of treated to control	Per cent reduction	Control	Treated	Ratio of treated to control	Per cent reduction
Oct. '44	1182.4	1058.0	0.895									
Nov.	952.8	23.0	.024	97.6	1174.5	1.0	0.001	99.9				
Dec.	371.0	109.5	.295	70.5	266.5	4.0	.015	98.5				
Jan. '45	259.0	40.5	.156	84.4	116.6	6.5	.056	94.4	101.0	0.5	0.005	99.5
Feb.	363.4	54.5	.150	85.0	169.6	4.0	.024	97.6	145.0	4.0	.028	97.2
March	375.4	32.5	.087	91.3	175.6	1.5	.009	99.1	161.2	1.0	.006	99.4
April	338.2	31.5	.093	90.7	153.6	3.5	.023	97.7	140.2	2.5	.018	98.2
May	154.0	23.5	.153	84.7	80.6	3.0	.037	96.3	77.2	2.0	.026	97.4
June	302.0	18.5	.061	93.9	166.4	0.5	.003	99.7	158.2	0.5	.003	99.7
July	514.6	2.0	.004	99.6	240.6	0.0	.000	100.0	212.0	0.0	.000	100.0
Aug.	358.4	6.0	.017	98.3	152.6	1.0	.007	99.3	134.4	0.0	.000	100.0
Sept.	367.8	14.0	.038	96.2	117.2	0.5	.004	99.6	107.2	0.5	.047	95.3
Oct.	581.8	37.0	.064	93.6	159.4	2.5	.016	98.4	146.8	0.0	.000	100.0
Nov.	571.4	37.0	.065	93.5	179.6	6.5	.036	96.4	153.0	4.0	.026	97.4
Dec.	185.8	20.5	.110	89.0	90.4	9.0	.100	90.0	78.6	9.0	.103	89.7
Average	407.8	32.1	.079	92.1	231.6	3.1	.013	98.7	134.6	2.0	.015	98.5
Nov. '44-Feb. '45	488.1	56.9	.117	88.3	431.8	3.9	.009	99.1				
Mar.-June	292.1	26.5	.091	90.9	144.1	2.1	.015	98.5	134.2	1.5	.011	98.9
Jul.-Oct.	455.7	14.8	.032	96.8	167.5	1.0	.006	99.4	150.1	0.1	.001	99.9

<sup>1</sup> Pretreatment month.

show yet higher reduction percentages. For the third four-month post-treatment period (July-October, 1945) the reduction of blood-engorged *Anopheles* surviving twenty-four hours reaches the impressive figure of 99.9%. The possibility of malaria transmission in the houses of the treated village during this period becomes exceedingly remote.

Another point emphasized in this table is the progressively increased effectiveness of each successive treatment. If we examine under "All *Anopheles*"

the column for "Per Cent Reduction" we find 88.8% for the first four-month post-treatment period (Nov. '44-Feb. '45), 90.9% for the second period (Mar.-June '45), and 96.8% for the third period (July-Oct. '45). This is probably due to the fact that in each case a residue of DDT remains from the previous treatment, and the fresh treatment adds DDT to that already present.

TABLE 8'

*Average number of culicines per house, per month, based on semi-weekly catches totalling 20,742 specimens*

	ALL CULICINES				BLOOD-ENGORGED CULICINES				BLOOD-ENGORGED CULICINES SURVIVING 24 HOURS			
	Control	Treated	Ratio of treated to control	Per cent reduction	Control	Treated	Ratio of treated to control	Per cent reduction	Control	Treated	Ratio of treated to control	Per cent reduction
Oct. '44	245.6	224.0	0.912									
Nov.	194.4	10.5	.054	94.6	56.0	1.0	0.018	98.2				
Dec.	362.8	98.5	.272	72.8	76.0	8.0	.105	99.5				
Jan. '45	257.0	152.5	.593	40.7	86.8	20.0	.230	77.0	72.2	6.0	0.083	91.7
Feb.	259.6	67.0	.258	74.2	118.8	12.0	.101	99.9	100.6	7.0	.070	93.0
Mar.	264.6	16.5	.062	93.8	86.6	2.0	.023	97.7	82.2	2.0	.024	97.6
Apr.	339.0	24.5	.072	92.8	123.2	0.0	.000	100.0	121.2	0.0	.000	100.0
May	151.8	42.0	.277	72.3	66.8	7.5	.112	88.8	65.8	7.0	.106	99.4
June	210.8	18.0	.085	91.5	77.8	1.5	.019	98.1	75.2	1.5	.020	98.0
July	108.0	11.5	.106	99.4	49.8	0.5	.010	99.0	41.4	0.0	.000	100.0
Aug.	202.4	18.0	.089	91.1	65.0	1.0	.015	98.5	50.4	0.5	.010	99.0
Sept.	300.4	23.0	.077	92.3	82.6	2.0	.024	97.6	69.2	1.0	.014	98.6
Oct.	285.8	13.0	.045	95.5	78.4	0.5	.006	99.4	66.2	0.5	.008	99.2
Nov.	365.8	54.0	.148	85.2	130.0	5.5	.042	96.8	113.0	5.5	.049	95.1
Dec.	276.4	28.0	.101	99.9	94.0	13.0	.138	86.2	78.8	12.0	.152	84.8
Average	255.6	41.2	.161	93.9	85.1	5.3	.062	93.9	78.0	3.6	.046	95.4
Nov. '44-Feb. '45	268.5	82.1	.306	69.4	84.4	10.2	.121	87.9				
Mar.-June	241.6	25.3	.105	99.5	88.6	2.8	.032	96.8	86.1	2.6	.030	97.0
Jul.-Oct.	224.2	16.4	.073	92.7	69.0	1.0	.014	98.6	56.8	0.5	.009	99.1

<sup>1</sup> Pretreatment month.

For purposes of comparison, similar data for culicine mosquitoes are given in table 8. These will be found to confirm, in a general way, the results obtained with anophelines.

#### *Stable Trap Catches*

The data from the horse-baited stable traps placed in and around the treated village are of much interest. A full discussion of the relation of anopheline abundance to meteorological and other conditions cannot be given here, but there

are several gross features of importance for this experiment. We can see from table 9 and Chart II plotting the abundance of *Anopheles* that, in the area considered, there are well defined seasonal fluctuations; the period of minimum

TABLE 9

*Average nightly catches of Anopheles from horse-baited stable traps at Gatuncillo*

WEEK OF	TRAP #1	TRAP #2	TRAP #3	WEEK OF	TRAP #1	TRAP #2	TRAP #3
Oct. 8, '44	384	746	90	May 6	6	15	17
15	402	402	183	13	5	16	5
22	460	860	265	20	12	15	9
29	567	865	192	27	8	20	5
First DDT Treatment				June 3	16	16	3
Nov. 5	126	35	91	10	26	15	18
12	152	47	149	17	34	71	86
19	223	121	164	24	23	52	26
26	199	272	126	Third DDT Treatment			
Dec. 3	170	200	370	July 1	15	33	28
10	133	328	262	8	8	11	50
17	100	23	120	15	13	27	63
24	136	11	88	22	34	52	54
31	80	56	133	29	19	44	39
Jan. 7, '45	34	42	40	Aug. 5	19	17	12
14	64	53	145	12	59	51	108
21	135	79	126	19	168	56	79
28	125	44	106	26	82	55	66
Feb. 4	97	136	180	Sept. 2	74	35	89
11	138	88	104	9	185	99	216
18	212	178	70	16	62	81	58
25	188	No catch	130	23	91	64	74
Second DDT Treatment				30	145	67	141
Mar. 4	42	8	83	Oct. 7	144	166	104
11	109	22	86	14	345	483	171
18	54	20	61	21	245	352	154
25	25	5	22	28	284	678	298
Apr. 1	7	9	39	Nov. 4	229	918	249
8	17	12	21	11	432	652	260
15	26	133	18	18	84	387	157
22	38	36	11	25	148	737	210
29	9	91	21	Dec. 2	189	611	271
				9	78	139	166
				16	121	105	124
				23	78	103	86

numbers being in May, that of maximum numbers being at the end of the wet season, during October and November. Examination of the curves for the last several months of 1945, at a time when no DDT had been applied for a number of months (the last treatment having been at the beginning of July) shows that the

natural drop in numbers occurs during the latter part of November or in December, coincident with the end of the wet season and flood conditions. If we consider then the portions of the curve for the last months of 1944 we note a decided modification of this natural trend. Examining first Gatuncillo stable trap number two, located in the center of the treated village, we note a dramatic drop from a level of 402-865 *Anopheles* per night, in October, to a level of 35-47 per night during the weeks of November 5th and 12th. The drop is obviously a consequence of the DDT house spraying the week of October 29th. This spraying was performed at a time when the *Anopheles* were at their maximum

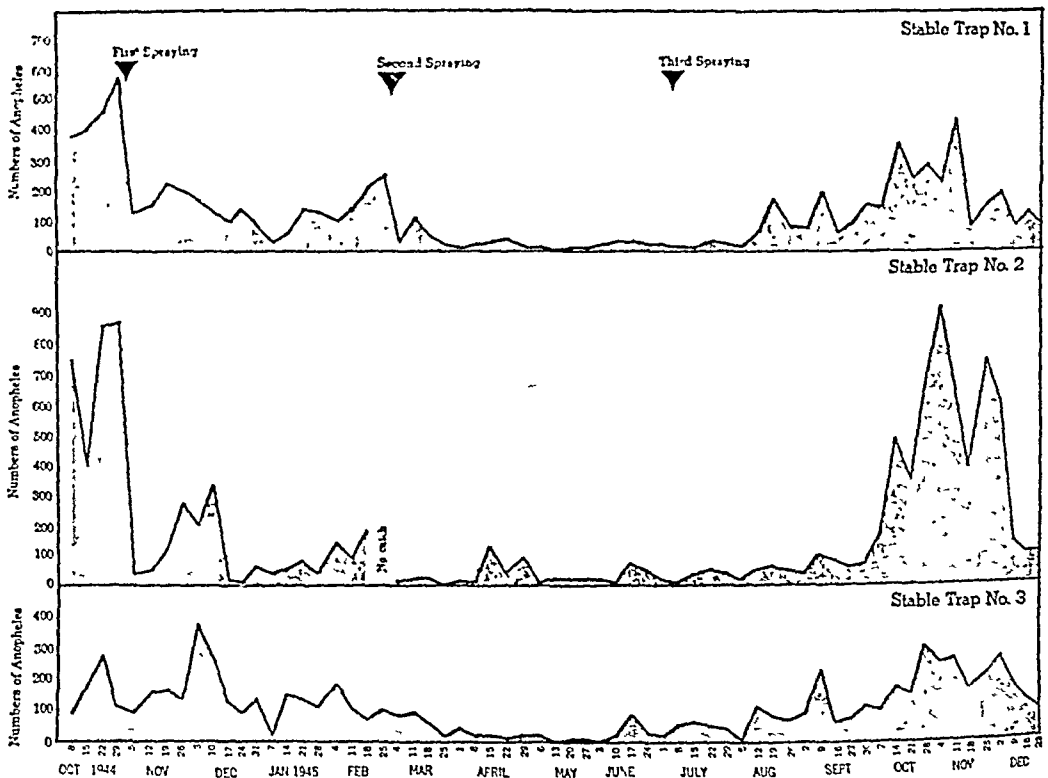


CHART II. CHART SHOWING ANOPHELINE MOSQUITO ABUNDANCE IN THREE STABLE TRAPS AT AND NEAR GATUNCILLO, PANAMA

numbers for the year, but in spite of this heavy population pressure, there was excellent control in the village area for two weeks. By the week of the 10th of December the population had recovered to the point where 329 *Anopheles* per night were taken. At this point the flood intervened, producing a sharp drop the following week. With the almost complete absence of rainfall after this point the population recovered only to the moderate level of abundance characteristic of dry season. There was unfortunately no catch made the week of February 25th, but the drop the following week, coincident with the second house spraying on March 1st and 2nd, is obvious. The drop at this time is more prolonged, persisting for six weeks. This may be due to the accumulative effect of a second spraying, the greater effectiveness of the spraying at a time

when anopheline population levels are small or moderate, or to an undetermined natural phenomenon. The third spraying of Gatuncillo on the 3rd and 4th of July came at a time when the anopheline level was low and little effect is apparent in the chart at this time. The normal expansion of the anopheline population in October and November of 1945 was apparently in no way inhibited by the prior sprayings.

Considering then the anopheline population of the village area outside the houses the following tentative generalizations may be made.

1. Even at times of great anopheline abundance a high degree of area control may be effected for a period of two to three weeks by a spraying of houses alone.
2. During a period of moderate anopheline abundance, effective control for six weeks following house spraying may result.
3. House spraying three months prior to a period of great anopheline abundance is wholly ineffective in preventing the normal increase of *Anopheles* in an area.

Having demonstrated that DDT spraying of houses may produce significant modifications in the anopheline population of the village area outside the houses, as well as in the houses themselves, we may now inquire into the effects of this treatment in the forest surrounding the village. An examination of the chart of anopheline abundance in Gatuncillo stable trap number one provides information on this point. This trap is located approximately three hundred feet north-east of the treated village (See aerial photograph, plate I, figure 1). While this trap does not catch such great numbers of *Anopheles* as trap number two, the general configuration of the chart showing captures in it is the same. The drop in numbers of *Anopheles* the week of November 5th, 1944 corresponds with that in trap number one, but is of lesser magnitude. In the month preceding treatment of Gatuncillo, October 1944, average nightly catches of *Anopheles* were from 384 to 567. For the two weeks following treatment catches dropped to 126 and 152. Again at the time of the second spraying a marked drop from 188 *Anopheles* per trap-night the week of February 25th, 1945 to 42 per trap-night the week of March 4th may be noted. As with trap number two, the mosquito populations at the time of the third spraying, July 3rd and 4th, 1945, were too low for any significant effect to be shown. The high anopheline populations of the following October and November are similar to those which appeared in trap number two.

From these data it may tentatively be stated that not only does house spraying in a village reduce the anophelines of the houses and the village area itself, but also significantly modifies the populations in the adjacent forest up to at least three hundred feet. House spraying alone, therefore, gives some measure of protection not only in the houses, and in the village streets, but in the forest immediately adjacent to the treated village. We may call this "peripheral effect".

The graph showing anopheline catches from stable trap number three, some nine hundred feet south of Gatuncillo (Chart II), may next be examined for further peripheral effects. It will be seen at once that there are no dramatic

drops in catches following the first two sprayings, as may be observed in the graphs for traps one and two. The peripheral effect apparently does not, therefore, extend for distances as great as nine hundred feet.

#### EFFECTS ON MALARIA

The effect on malaria incidence is the ultimate criterion of the success of the residual spray method. In the area here considered, where malaria is endemic, significant data from the malaria indices may be obtained only after a period of several years. Information which we now have, however, covering the fourteen-month period since the first spraying, is very suggestive of a significant downward trend in malaria incidence in the treated village.

TABLE 10

*Comparison of malaria rates at three Chagres River villages, based on the cumulative rate in individuals examined either five or six times during the year*

	SANTA ROSA AND GUAYABALITO	GATUNCILLO	DIFFERENCE
<sup>1</sup> 1940-41	45.6%	33.3%	-12.3%
<sup>1</sup> 1941-42	52.8%	60.9%	+8.1%
<sup>1</sup> 1942-43	39.7%	46.7%	+7.0%
<sup>1</sup> 1943-44	42.5%	45.5%	+3.0%
	Untreated	Treated	
<sup>2</sup> 1944-45	51.3%	24.0%	-27.3%
<sup>3</sup> 1945	52.0%	14.8%	-37.2%

<sup>1</sup> Annual rate based on the year September to August.

<sup>2</sup> Annual rate based on the year November to October.

<sup>3</sup> Annual rate based on the year January to December.

Malaria indices given here are based on the bimonthly thick blood-film surveys made by Dr. H. C. Clark and his technicians. These surveys are made each February, April, June, August, October and December. For convenience the "year" used by Dr. Clark is from September to August. Since the first spraying of Gatuncillo was performed at the beginning of October 1944, we have broken down the data from the bimonthly surveys and here present the indices for the one-year period November 1944 to October 1945 (table 10). This is the twelve-month period immediately following our first spraying. We have also extracted the data necessary to calculate the indices for the twelve-month period January to December 1945, the year commencing two months after the first spraying. The use of this last mentioned period eliminates positive blood-films occurring in the period immediately following the first spraying, i.e., malaria contracted prior to the first village treatment. The decline of the malaria index at Gatuncillo, in the period following treatment, is striking, the cumulative index being 14.8% for 1945 as compared with 52.0% for the same one-year period at the control villages of Santa Rosa and Guayabalito.



These indices for the 1945 period are unfortunately based on relatively small numbers of individuals, as but twenty-seven persons were examined either five or six times during the year at Gatuncillo, and seventy-nine at Guayabalito and Santa Rosa. While we have survey data from three to four times as many individuals, those persons examined less than five times during the year have been eliminated, since by their absence from the villages at the time of surveys they are disqualified as continuous residents of the houses with which we are concerned.

One difficulty in attempting to establish, by an examination of the parasite index, whether or not malaria transmission has been stopped, arises from the uncertainty as to whether a positive thick film represents a relapse from previously contracted malaria, or a fresh infection. In the villages here studied persons positive on blood thick film examination are treated with a five day course of atabrine of 0.1 gram, three times a day. (See Clark and Komp, 1941, and Annual Reports of the Gorgas Memorial Laboratory.) It has been indicated by McCoy (1945) and others that *falciparum* is less likely to relapse than *vivax*

TABLE 11

*Comparison of falciparum malaria rates at three Chagres River villages, based on the cumulative rate in individuals examined either five or six times during the year*

	SANTA ROSA AND GUAYABALITO	GATUNCILLO	DIFFERENCE
	(Control)	(Treated)	
<sup>1</sup> Nov. 1944–Oct. 1945	39.7%	12.0%	–27.7%
<sup>2</sup> Jan. 1945–Dec. 1945	39.2%	7.4%	–31.8%

<sup>1</sup> Twelve month period immediately following first spraying.

<sup>2</sup> Twelve month period commencing two months after first spraying.

following the administration of atabrine. It is therefore of interest to extract the index for *falciparum* malaria alone for the period following the first village spraying. These data are presented in table 11. The difference between the indices in the control and treated villages is even more marked than that noted in the preceding table which considers all forms of malaria together.

A more detailed analysis of the effects of the residual spraying on the malaria in these villages is in order, and will be made by Dr. H. C. Clark at a later date.

#### COST ESTIMATES

The materials used in the present experiment were DDT and kerosene. Based on the experience of several treatments we find that approximately three gallons of solution are used per house. This represents approximately a pound and a quarter of DDT per house. The cost of DDT has undergone great reduction in the period since this experiment began, when limited quantities for experimental use only were available. Stierli *et al.* (1945) use \$0.64 per pound in calculating costs, but recent information from the Office of the Surgeon General, U. S. Army, indicates that the current price in the United States, less overseas transportation costs, is \$0.46 per pound. The actual cost delivered to the various Caribbean

countries will vary with transportation costs and possible duties. For purposes of our calculations we will use the arbitrary figure of \$0.50 per pound. This should be a fair estimate of the cost of DDT in this area, at the time this report becomes available. Kerosene for this experiment was locally obtained at \$0.07 per gallon from the oil storehouse of the Panama Canal. The cost of materials for the treatment of each house is thus approximately \$0.84: \$0.21 for three gallons of kerosene and \$0.63 for a pound and a quarter of DDT.

To treat twenty-five houses we found it took two laborers and a foreman two days. Laborers received \$1.25 a day, the foreman \$3.33 per day. The total cost of labor to treat twenty-five houses was thus \$11.66 or \$0.47 per house. The combined cost of materials and labor was \$1.31 per house. For the three treatments per year here recommended the cost per house, per year is \$3.93.

There are several factors which influence this cost estimate.

1. The labor cost per house is high, since we treated only one relatively small village, and considerable time was expended in instructing operators how to apply the solution at a uniform rate. The same amount of labor, once proficient, and routinely engaged in work of this sort, could accomplish much more in a day and substantially reduce labor costs.

2. With the cane and thatch construction present in the village, spraying outside as well as inside houses was desirable. Elsewhere in this region where solid wall construction is used it would be necessary to spray only the interiors. This would substantially reduce the costs of both labor and material.

3. As we have noted previously the area of our experiment is one where the most difficult conditions exist since anopheline production proceeds throughout the year, even in the height of the dry season. In western Panama and elsewhere, where dry season conditions of three or four months seriously inhibit *Anopheles* production, it is probable that two treatments per year, strategically timed at the beginning and middle of the rainy season, would provide protection of as high an order as that obtained on the Chagres River with three treatments. The annual cost per house might thus be reduced by one third.

4. The equipment used in this experiment was Army issue and its cost and depreciation is not included in the cost estimate since data of this sort are not available. Were a long-term large-scale program of DDT residual house spraying set up, however, the initial cost of equipment would not add substantially to the per-house cost of the work.

5. In a large-scale program over a considerable area transportation cost would be introduced.

#### DISCUSSION

Of the non-naturalistic methods, there are two classes of mosquito control for reducing malaria transmission now universally employed. They are spoken of as permanent and temporary. Permanent control consists of such measures as drainage of breeding areas in a radius a mile or so about the area to be protected, and screening dwellings. These are relatively expensive measures but quite justified in areas of concentrated populations, as large towns and cities, particularly where individual incomes are substantial. Temporary measures

such as larviciding are less expensive but must be frequently repeated. In small rural communities and particularly in areas of scattered individual houses the cost of this work is often virtually prohibitive. It is also difficult to supervise work of this sort.

The DDT residual spraying of dwellings essentially meets the needs of this latter situation, providing the population is a relatively domestic one. The tendency to kill selectively the engorged, and hence potentially infected mosquitoes, makes for great efficiency. The technique tends to bring malaria control a step beyond "species control" to "*infected mosquito control*". The relatively low cost of the method makes malaria control possible among the poorer classes, those most needing it, since they now receive little or no protection, and constitute the main "seed bed" of the disease. It is an ironic but fortunate circumstance that DDT residual treatments will work best where the people are poorest. The lower income classes in the American tropics cannot afford and do not use paint on their dwellings. A characteristic of DDT is that it is far more effective, both initially, and in its residual effects, on unpainted surfaces. Since this low income group constitutes the main "seed bed" of malaria, effective control at this level will give added protection to the higher income classes, as the main source of mosquito infection will have been eliminated.

There is a psychological factor, wholly irrelevant to the malaria control picture, but nevertheless of great importance. The rural inhabitant of the American tropics with a meager education is ill equipped to appreciate the nature of malaria transmission, and it is difficult to impress him with the value of such measures as larviciding. DDT residual spraying of dwellings produces not only an immediate and obvious reduction of *Anopheles* in his home, but reduces the annoyance from pest mosquitoes as well (See tables on culicine mosquitoes). In addition, such household pests as cockroaches, ants, bedbugs, and wasps are markedly reduced. It was our experience that while there was at first considerable scepticism and some objection to the first treatment of the experimental village, the inhabitants welcomed retreatment and were cooperative in every way thereafter. A favorable psychological attitude of this sort facilitates operations and provides a body of public opinion which will tend to approve expenditure of public funds for the work.

#### SUMMARY AND CONCLUSIONS

This paper reports the results of the first fifteen months study of the DDT residual house spray method in the middle Chagres River area of Panama, where the principal malaria vector is *Anopheles albimanus*.

Houses of cane wall and palm-thatch roof construction at the village of Gatuncillo were sprayed, inside and out, with a 5% solution of DDT in kerosene at four-month intervals (excepting one trial period of six months). Entomological observations were made at this treated village and two adjacent villages used as controls.

It is demonstrated that with this technique anophelines visiting the dwellings are affected in three ways:

1. There is a large reduction in numbers of mosquitoes.

2. Among the mosquitoes which are taken in treated dwellings there is a marked reduction in the per cent engorged, since DDT activates the insects and they lose interest in feeding.

3. Among the engorged mosquitoes the twenty-four hour survival rate is low for three months after treatment.

It is thus indicated that the technique tends selectively to reduce the malaria transmission potential by affecting principally those mosquitoes concerned in transmission.

Four months is established as the optimum time for retreatment in the area studied.

It is demonstrated that with each successive treatment the degree of control improves. (For the period following the third treatment the malaria transmission potential is reduced 99.9%.)

Treatment of houses alone was found to produce marked reduction of mosquitoes in the village area outside the houses, and even to some measure in the forest adjacent to the village (for at least three hundred feet), for several weeks.

There is evidence of reduction of malaria in the fourteen-month period following the first treatment.

The cost of the treatment under conditions of this experiment is calculated as \$1.31 per house. Factors affecting the cost of applying the method elsewhere in tropical America are discussed.

The place of this method in relation to other malaria control procedures and to the economy of the area is discussed.

#### ACKNOWLEDGMENTS

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# EPIDEMIC ENCEPHALITIS IN THE FAR EAST

## A REVIEW

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### I. INTRODUCTION

It is the main purpose of this paper to present a summary of the existing knowledge of the epidemic encephalitides occurring in the Far East. These diseases are relatively unfamiliar to the majority of American physicians. Since the chances of their being encountered in portions of the eastern Asiatic continent seem likely, and, since the possibility that they may be introduced into the United States must be considered, it was thought that a description of their epidemiology, clinical manifestations, and diagnosis would be of interest. Two types of acute encephalitis caused by filterable viruses are endemic in the Far East; these are Japanese B encephalitis and Russian spring-summer encephalitis.

Most of our present knowledge regarding Japanese B encephalitis dates from 1928 when a comprehensive paper by Kaneko and Aoki appeared (1), in which these authors concluded that the acute epidemic encephalitis occurring in Japan in the late summer and autumn was a distinct clinical entity and was to be differentiated from encephalitis lethargica (von Economo's disease). Since both forms occurred in Japan they designated encephalitis lethargica as Type A and the newly described syndrome as Type B encephalitis. The latter has also been called "summer cerebrospinal fever" and "atypical poliomyelitis of Japan." Since 1934 the etiologic agent of this disease has been repeatedly isolated by Japanese workers and it is probable that occasional isolations were made prior to that time (1, 2).

In the Russian literature there is described a disease which occurs in the autumn months in eastern Siberia and which is termed "autumn encephalitis" (3). (This is not to be confused with Russian spring-summer encephalitis.) Most evidence points to the fact that in all probability Russian autumn and Japanese B encephalitis are identical syndromes. They have a similar epidemiology and clinical course, and the pathological changes produced by each are likewise indistinguishable. In this review no differentiation will be made between these diseases. Certain cross-immunity studies, however, present some conflicting evidence regarding the synonymy of these two diseases, and before it can be stated with certainty that they are caused by the same filterable virus, further research is indicated.

In eastern Russia and Siberia there occurs another acute encephalitis, primarily affecting workers in the forests which has been named "spring-summer" or "verno-aestival encephalitis" because of its pronounced seasonal nature.

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The infection also appears in the literature as "tick-borne encephalitis" and "wood-cutters' disease." The causative agent of this disease is also a virus and was discovered by Silber in 1939 (4).

## II. JAPANESE B ENCEPHALITIS

### A. Epidemiology:

**Incidence:** It is likely that the Japanese Islands have been endemic areas for acute viral encephalitis since 1870 and probably before. In certain prefectures the summer months were invariably accompanied by localized outbreaks and occasionally isolated cases of acute encephalitis or meningo-encephalitis.

TABLE 1  
*The incidence of Japanese B encephalitis in Japan from 1924 to 1937*

YEAR	TOTAL CASES	CASES PER 100,000	TOTAL DEATHS	DEATHS PER 100,000	FATALITY RATE
1924	6,125	10.4	3,797	6.4	62.0%
1925	139	0.2	69	0.1	49.6%
1926	864	1.4	583	0.9	67.5%
1927	1,006	1.6	716	1.1	71.2%
1928	72	0.1	50	0.07	69.4%
1929	2,058	3.1	1,340	2.0	65.0%
1930	499	0.8	360	0.58	72.14%
1931	129	0.2	97	0.15	75.19%
1932	689	1.0	391	0.57	56.46%
1933	791	1.2	510	0.77	64.48%
1934	278	0.4	171	0.25	61.51%
1935	5,370	7.8	2,264	3.3	42.16%
1936	1,305	1.9	696	1.0	53.33%
1937	2,030	2.8	1,115	1.5	54.93%
Total .....	21,355		12,159		56.9%

From Matheson Commission Report III, page 159, 1939.

Sufficient cases to warrant the description "epidemic" occurred in 1873, 1901, 1903, 1907, 1909, 1912, 1916, 1917, 1919. In 1924 an outbreak of cases occurred in mid-summer, creating panic in the inhabitants of Central Japan. More than 6,000 clinical cases with nearly 4,000 deaths were recorded within six weeks, and Kaneko and Aoki have aptly described it as a "blitzschlagähnliche" epidemic. Table 1 is a compilation of the morbidity and mortality of Japanese B encephalitis in Japan from 1924 to 1937, the last year for which reliable figures are available.

No information has come to the writer's attention regarding the incidence of encephalitis in Japan since 1940. It will be noted that even in mild or non-epidemic years the fatality rate of Japanese B encephalitis is formidable. The morbidity and fatality rate figures prior to 1924 may not be entirely accurate, and are therefore not included in Table 1. In recent years, owing to more accu-



rate diagnosis together with the stricter enforcement of ordinances requiring the reporting of cases, the epidemiological reports of Japanese B encephalitis are more trustworthy.

There is a difference of opinion regarding the validity of the high fatality rate. It is claimed by some (1, 6) that because of mild undetected cases of Japanese

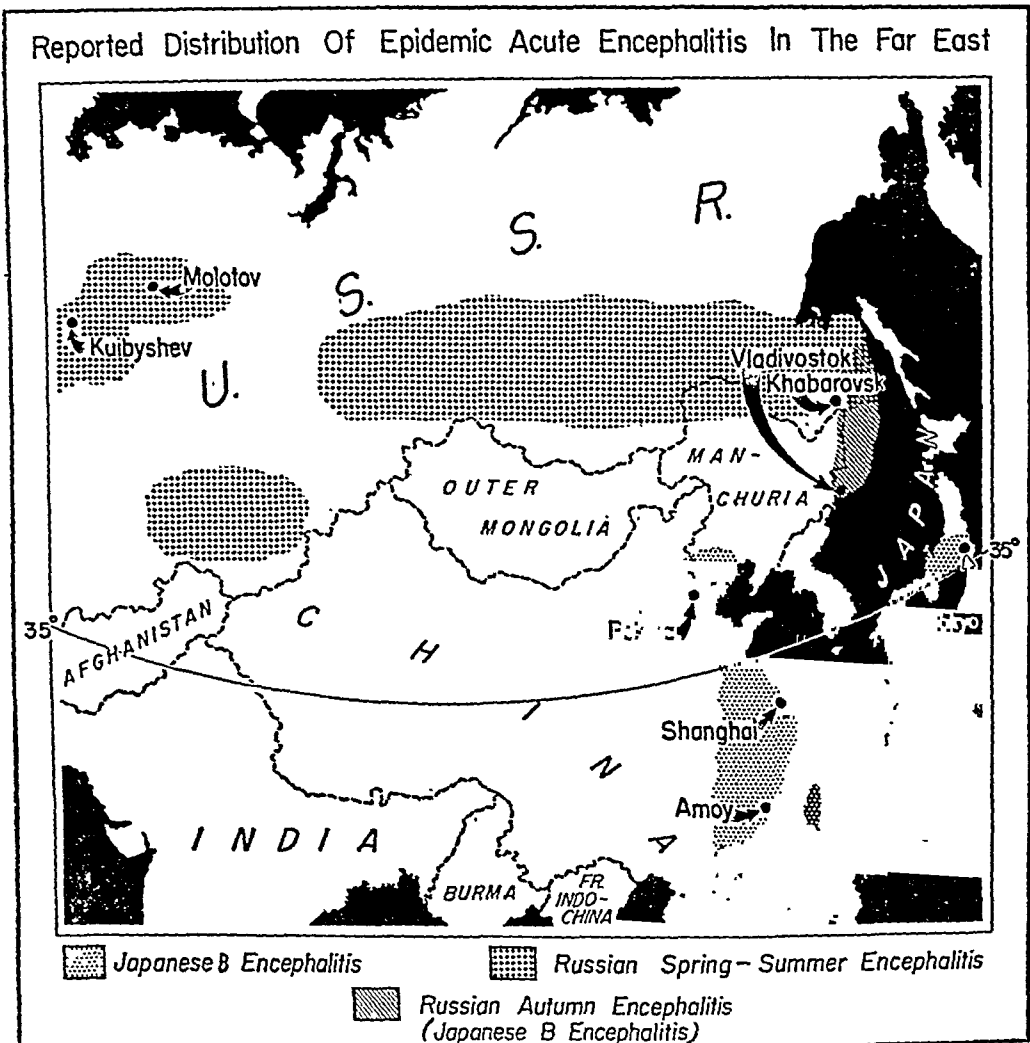


FIG. 1

B encephalitis, particularly in the young, the fatality rate is lower than it actually appears. The demonstration of the presence of virus neutralizing antibodies in many Japanese who have never had clinical encephalitis lends support to this view.

Season and climate: Japanese B encephalitis is a disease of the summer and early autumn. In Japan the daily temperatures reach a maximum at the end of July and August, and it is interesting to note that the morbidity peaks in epidemic

years have usually occurred at this time. The hotter the season the more likelihood of an epidemic. When epidemics occur they do not strike all areas simultaneously but usually originate in the southwest (6).

Geographical distribution: Japanese B encephalitis, in addition to Japan proper, has been observed in Siberia, Korea, Ryukyu Islands, Formosa, China, especially in the vicinity of Peking, and in the Maritime Krai. The disease has never been reported in the Philippine Islands (fig. 1).<sup>2</sup>

The massive epidemics of Japanese B encephalitis have been restricted to the Japanese Islands and exhibit a rather sharp geographical localization, even within these confines. The provinces bordering on the Inland Sea, just below the

TABLE 2

*Japanese encephalitis morbidity and fatality rates for the 1924 epidemic by prefectures*

PREFECTURE	ONSET OF EPIDEMIC	CASES	DEATHS	FATALITY RATE
Kagawa.....	Beginning August	1,973	1,189	60.2%
Kyogo.....	Ending August	826	545	65.9%
Toyama.....	Ending July	712	438	61.5%
Okayama.....	Beginning August	656	462	70.4%
Tottori.....	Mid July	423	210	49.6%
Tokushima.....	Beginning August	324	222	68.5%
Hiroshima.....	Mid August	279	203	72.7%
Ihime.....	Mid August	263	148	56.2%
Nagano.....	Beginning August	202	100	49.5%
Akita.....	Beginning September	156	94	60.2%
Yamaguchi.....	Mid July	145	89	61.3%
Fukuoka.....	Beginning August	121	59	48.7%
Shimane.....		108	74	68.5%
Aomori.....		103	61	59.2%
Kochi.....	Ending July	101	11	10.8%
Remaining 32 provinces .....		559	264	47.2%
All Japan.....		6,949	4,169	59.93%

Kaneko and Oaki: *Ergebn. d. Inn. Med. U. Kinderheilkunde*, 1928, 34: 342.

35th parallel, north latitude, have consistently been the chief foci and are among the hottest areas of Japan. Encephalitis of all types is less frequent in north-eastern Japan, a comparatively cool region during the summer months. The following table (table 2) is illustrative of the case distribution by prefectures in the 1924 epidemic and demonstrates the wide variation occurring in prefectures which may be in close proximity to one another.

In the Maritime Districts (fig. 1) adjacent to the Sea of Japan, Japanese B encephalitis begins at the end of August or during the middle of September, which is a few weeks after the usual appearance of the disease in Japan. As in the case of outbreaks in Japan, the Siberian outbreaks are usually preceded by extremely hot and dry weather (3).

<sup>2</sup>Pette (7), on the basis of the pathological findings alone, claims two fatal cases of Japanese B encephalitis have occurred in Germany.

The disease in Siberia, especially Khabarovsk, is primarily rural in its distribution and rare in the larger settlements or towns. Geographically it occurs on the steppes, barren plains and in the vicinity of lakes, swamps or marshy pools where the water is relatively stagnant.

Up until 1934 there had been few reported observations of encephalitis in China. Pfister (8) collected records of one hundred cases predominantly occurring in the winter months prior to 1929. These cases bear a closer resemblance to encephalitis lethargica than to Japanese B. In Table 3 are tabulated the more recently recorded cases of acute epidemic encephalitis in China

TABLE 3

*Reported cases of encephalitis probably due to Japanese B virus in China, 1934-1941*

YEAR	PLACE	CASES	DEATHS	REPORTED BY	REMARKS
1934-1935	Peking	2	0	Kuttner & Tung	One convalescent serum neutralized Japanese B & St. Louis viruses. One convalescent serum neutralized Japanese B. Not tested for St. Louis encephalitis.
1935	Amoy	5	?	Su	Occurred simultaneously with the epidemic in Japan.
1937	Shanghai	5	0	Loewenburg	Clinical description only. No laboratory studies.
1938	Peking	13	3	Chu, Wu & Teng	Neutralizing antibodies for Japanese B virus developed in six of eight tested in both series. Three had antibodies when first examined.
1939	Peking	16	?	Huang	
1941	Peking	1	1	Yen	Fatal case in child. Virus isolated from brain was neutralized by Japanese B antiserum.
Total .....		42			

which, on the basis of seasonal occurrence, clinical findings and in some cases serology, are probably due to Japanese B virus.

It is possible that mild or inapparent infections occur to a far greater extent than the above figures would indicate. In Formosa this disease recurs each summer with a somewhat higher incidence in children than is found in Japan (6).

Age, sex, and occupational distribution: Although inapparent infections among the younger people may be responsible for the seemingly low morbidity in younger age groups, considering only frank cases, the number of patients with recognizable central nervous system symptoms increases with age. The incidence and fatality rate in this infection increases markedly in persons over 40 years (table 4), in which respect it resembles St. Louis encephalitis occurring in the United States.

Iimura (6) finds no variation in this adult hypersusceptibility in epidemic and in inter-epidemic periods.

Japanese B encephalitis occurs somewhat more frequently in males than in females (1, 6). In 10,952 cases from 1924 to 1933 the sex ratio of cases, when corrected for the ratio of males to females in the population, was 124 males to 100 females.

The disease is not associated with any particular occupational group. Early reports (15) claimed that farming peasants were the most frequent sufferers. In contrast Iimura in 1936 reported a preponderance of cases in the higher economic levels, with the greatest morbidity among "intellectual" workers. In the Tokyo epidemics, Inada found all social strata and occupations to be afflicted equally.

TABLE 4  
*Age distribution of Japanese B encephalitis in Japan from 1924 to 1933*

AGE GROUP	CASES	CASES/100,000 OF CORRESPONDING AGE GROUP	DEATHS	DEATHS/100,000 OF CORRESPONDING AGE GROUP	FATALITY RATE
0-10	1,450	9.6	725	4.8	50.0%
11-20	1,221	9.7	618	4.9	50.6%
21-30	877	9.3	476	5.0	54.2%
31-40	841	11.7	468	6.5	55.7%
41-50	1,060	16.9	589	9.5	55.6%
51-60	1,805	40.6	1,187	26.7	65.8%
61-70	2,335	81.1	1,675	58.2	71.7%
71-80	2,127	147.5	1,770	122.7	83.2%
81-90	495	183.3	413	152.9	83.4%
91-100	24	168.2	19	133.1	79.4%
Total .....	12,235		7,940		64.9%

From Matheson Commission Report III, page 162, 1939.

Racial predisposition is not a determining factor. Orientals and Europeans are equally susceptible to this disease. Fatigue and undue exposure to heat and sunlight, may be contributing influences, although as regards the latter, Hashimoto and his associates (16) state that the 1935 Tokyo epidemic continued through cooler weather.

While multiple cases in families do occur, their number is small, and certainly insufficient to support adequately a thesis of contact infection. Cases in physicians and nurses are rare. In Kaneko's clinic there were 54 cases during the 1924 epidemic and even though no masks or gowns were worn, no infections occurred in the medical or nursing staff.

In summary, Japanese B encephalitis is a disease which is fatal in approximately half its victims. The hot months of summer favor its propagation and the clinical cases predominate in older age groups. Occurring regularly in certain endemic locales, the disease victimizes those in all walks of life.

*B. Vectors:* It was formerly contended, particularly by Japanese workers (17), that Japanese B encephalitis was spread through contact with infected droplets, possibly originating in healthy carriers. While this thesis accounts in some measure for the epidemiological behavior of this disease, it was superseded in 1937 by the theory postulating mosquito transmission (18).

The grounds for regarding this encephalitis as a mosquito-borne disease may be briefly stated: (1) Virus is present in the peripheral blood of mice and infected rodents and thus is accessible to mosquitoes (18). (2) When *Culex tritaeniorhynchus*, *C. pipiens*, var. *pallens*, and *Aedes togoi*, common Asiatic species, were allowed to feed on infected mouse brain they were readily infected (18, 19, 20). (3) The virus of Japanese B encephalitis has been isolated from mosquitoes caught in the field. Russian and Japanese workers were able to isolate the virus from wild mosquitoes taken from epidemic areas but not from the same species simultaneously trapped elsewhere (19, 20). Groups of wild mosquitoes consisting of *Culex pipiens*, *C. tritaeniorhynchus* and "*Culex* and *Aedes*" were shown to be infected when inoculated into mice (20). Although the Russian workers were not able to produce the disease in mice by the bite of naturally infected mosquitoes, Mitamura and associates claim to have accomplished this repeatedly. (4) Mosquitoes once infected remained so for life and the Japanese have reported the presence of virus in ova of infected mosquitoes although adults developing from such ova were found not to be infected (18). This, however, requires more confirmation before being accepted. The species of mosquitoes most susceptible to experimental infection are *C. pipiens*, var. *pallens*, *Aedes togoi*, and *A. csoensis*. These species are particularly prevalent during July and August in Japan, Formosa, the Maritime Krai and parts of China and Siberia. It is worthy of mention that experimental transmission of this virus has been accomplished in species of mosquitoes indigenous to the United States (21). These were *Culex pipiens*, *C. quinquefasciatus*, *Aedes nigromaculis*, *A. lateralis*, and two species of *Culiseta*. Although, so far as the author is aware, Japanese B encephalitis has never been reported as naturally occurring in man or animals in this hemisphere, the possibility of its accidental or deliberate introduction must be reckoned with.

The natural reservoir for this virus in winter and non-epidemic periods is still unknown. A report by Mitamura and his associates (22) claimed that they had demonstrated virus in the blood of dogs in Tokyo during July, August, and November. These animals were brought from Hokkaido, a non-infected area, to Tokyo in February. They were kept under observation in this endemic region for nine months and their blood was examined periodically for the presence of virus and neutralizing antibody. According to the Japanese workers virus first appeared during July, August, or November in the blood of six out of nine dogs tested. No statement is made as to how long this virus persisted. Furthermore, neutralizing antibodies appeared in these animals before or simultaneously with the finding of circulating virus. No mention is made as to titer of virus or antibody or whether these dogs were continuously exposed to mosquitoes.

Other domestic animals are at present implicated as natural reservoirs but only because antibody to the Japanese B virus has been demonstrated in their blood.

Further studies must be performed before knowledge of the natural reservoir of this disease is complete.

*C. Clinical Description:* Differential clinical diagnosis in any of the acute encephalitides is a difficult task and the literature contains no information which would permit the clinician to diagnose an isolated case of Japanese encephalitis as such without the assistance of the laboratory. The following description of the disease is gleaned from Kaneko and Aoki, Inada, and Hashimoto, Kudo and Uraguchi.

The incubation period of Japanese B encephalitis is approximately six to eight days. It may strike with explosive abruptness or it may follow a prodromal episode of malaise, headache and chills. The temperature raises rapidly to approximately 104 degrees Fahrenheit and is usually accompanied by any or all of the symptoms of meningo-encephalitis. Headache, vomiting, and rigidity of the neck and upper extremities are frequent. The patient's sensorium may range from the stuporous and apathetic to the violently delirious. Coma is frequent. As the disease progresses in its involvement of the central nervous system, death when it occurs usually ensues within the first week after onset.

The common neurological findings are rigidity of the neck and extremities; the exaggerated deep reflexes found early in the disease are later diminished or lost. Ocular disturbances such as photophobia, nystagmus and diplopia are not uncommon but far less frequent than in encephalitis lethargica. Tremors of the tongue and hands as well as aphasic speech disturbances were reported by Hashimoto, Kudo and Uraguchi in each of five cases occurring in Europeans and appear to be very commonly observed (1).

The Kernig sign and the Babinski reflex are only occasionally elicited.

Fever remains elevated for variable lengths of time, usually falling by lysis on the fifth to seventh day in those patients who recover.

Some disagreement exists as to the changes detectable in the chemistry of the spinal fluid. The pressure is increased to 200-300 millimeters of water, and there is almost always a leukocytosis of from 50 to 700 cells with much variation in the differential count. There is an increased protein and normal sugar concentration in the spinal fluid.

Japanese B encephalitis infrequently results in extensive or prolonged sequelae. Parkinsonian symptoms are extremely rare. In 2,000 surviving cases studied by Kaneko and associates (23) the percentage of survivors exhibiting sequelae of any description was only 3.1%.

As already mentioned, there is considerable evidence for the existence of cases of Japanese B encephalitis which are subclinical in their manifestation. Huang and Liu (24) report the case of a patient with a mild malaise, no cells in the spinal fluid, and only a transient stiff neck. Nevertheless, this patient developed specific neutralizing antibodies during the next few weeks.

*D. Pathology:* The pathological changes resulting from infection with the Japanese B encephalitis virus are almost entirely limited to the central nervous system and are similar to those observed in other types of epidemic encephalitis. They are of little differential diagnostic value.

There is a moderate amount of congestion of the meninges and the meningeal capillaries. The grey matter of the cortex, basal nuclei and pons appears slightly pinkish on section, and there is congestion of the intracerebral vessels.

Histologically, the vessels of the entire brain exhibit cuffs of mononuclear cells. Mild degenerative changes are found within the nerve cells of any region of the brain or cord; small foci of necrosis and even abscess formation have been reported. These are sometimes visible to the naked eye.

Taniguchi et al. (25) describe "elementary bodies" in the epithelium of Giemsa-stained infected rabbit cornea. However, this work has not been confirmed and filtration studies would indicate that the agent is much smaller than Taniguchi's description would indicate.

*E. Laboratory diagnosis:* The laboratory diagnosis of Japanese B encephalitis comprises either or both of two procedures. (1) Isolation and identification of the virus. (2) Demonstration of the appearance of specific antibodies for this agent in the patient's serum.

The virus is readily isolated from the brain and in contrast to most of the other neurotropic viruses (with the exception of the Russian spring-summer and lymphocytic choriomeningitis viruses) may be isolated from the blood and spinal fluid and it is sometimes detectable in the urine.

The method of choice for the isolation of the virus of Japanese B encephalitis is the intracerebral inoculation of Swiss mice. The resistance of adult mice (25.0 grams or more) to the peripheral inoculation of Japanese B encephalitis virus affords a rapid method for differentiation of the latter from the mouse adapted Russian spring-summer virus which will infect mice of any age when inoculated peripherally. According to Kasahara (26) it is often necessary to resort to "blind passage" for successful isolations. Other species readily susceptible to Japanese B virus are the Syrian hamster, monkey and sheep. Guinea pigs, rabbits, and chickens have little or no susceptibility to this agent as contained in human tissue.

By placing infected tissue in 50% buffered glycerin the virus can be preserved for at least three months. A more satisfactory method, when feasible, is to freeze the material in a gas-tight container in solid carbon dioxide. Under these conditions the agent is viable for at least a year.

It is not the purpose of this review to describe the symptoms which are produced by the virus when it is inoculated into experimental animals. These are described at length in such a standard text on virus diseases as van Rooyen and Rhodes (27).

Antibodies for Japanese B encephalitis are usually demonstrated by the neutralization test. This consists of a comparison of the titer of a standard virus in serum known to be free of antibody with the titer of the same virus in the presence of the serum sample under study. The complement fixation test, employing infected mouse brain as an antigen, while not yet applied to the diagnosis of human infection, has been used successfully in the measurement of antibody in experimental animals and may prove to be a useful technique in human diagnosis.

The interval between the onset of disease and the first appearance of antibodies

is not known exactly. Kawamura states that 29 of 32 patients had antibodies three months after the fever had dropped, but they were not tested prior to this date (17). Three of Huang and Liu's (24) cases developed neutralizing antibodies within three weeks (13, 14 and 21 days) but it is probable that antibody development occurs somewhat earlier and may be present in low titer shortly after onset. Antibodies have been shown to persist for five years or longer (2a).

It is not possible to state what titer of antibody is a prerequisite for making a positive diagnosis since a certain proportion of the normal population, especially in endemic areas, already exhibits levels which are within diagnostic range, i.e., neutralization of 100 or more minimal lethal intracerebral doses of virus (28). To circumvent this difficulty it is accepted practice to compare the antibody titer in serum taken (1) as soon as a diagnosis of encephalitis is entertained, and (2) in convalescence (three or more weeks after onset). Any significant increase in the amount of specific protective antibodies in the serum appearing during this interval is regarded as evidence of the disease.

*F. Therapy, vaccination, and control:* There is no specific therapy for this disease and all treatment is symptomatic.

A vaccine has been developed (29) from infected mouse brain inactivated with formaldehyde. Such a vaccine is effective in producing neutralizing antibodies in man and resistance to peripheral infection in mice (29). Further tests of this vaccine are in progress. Large scale vaccination of susceptibles in endemic areas of Siberia using a mouse brain vaccine has been advocated and practiced for the past five years (20, 30). Smorodintseff has reported two cases among 10,485 vaccinated persons as compared with 59 cases in 8,030 non-vaccinated personnel.

Vaccination procedures should be considered for persons residing in endemic areas or in the event of a generalized outbreak of Japanese B encephalitis.

The accepted mosquito control measures should be practiced, i.e., the use of suitable repellants and nets, and the spraying of human and animal dwellings with the insecticidal aerosols. Since the species of mosquitoes involved in the transmission of this infection breed mainly in pools and small collections of stagnant water, all buckets, cans, barrels, etc. should be drained. Larvicidal compounds should be used on larger bodies of water.

### III. RUSSIAN SPRING-SUMMER ENCEPHALITIS

*A. Epidemiology:* Rapid clarification of the etiology of Russian spring-summer encephalitis has occurred since 1939. The infection in man has probably existed since the first colonization of the forested regions of northern Russia and Siberia. Cases of encephalitis occurring in such areas during April, May and June were usually described as encephalitis lethargica, polioencephalitis, or encephalitis of unknown origin. In 1935 the clinical similarity of such cases as they occurred in the collectives was recognized and the disease was established as an entity (31). It has also been called "tick-borne encephalitis", "woodcutter's encephalitis", and "forest-spring encephalitis."

The disease occurs in a more or less continuous band extending across the northern Siberian and Russian territories. It is present in the Khabarovsk and



Maritime Krai, at Molotov in western Siberia, near Leningrad, and in the Karelian Isthmus, White Russia, and in the region of the lower Volga (the District of Kuibyshev) (fig. 1). More recently cases have been reported in the Kazakh Republic of west central Siberia. An important characteristic of Russian spring-summer encephalitis is its confinement to an endemic area from which it seldom spreads.

The victim of this disease almost invariably is one who has worked in the virgin forests. Lumbermen, linemen, road builders, hunters, surveyors, foresters, and sportsmen are usually encountered in the roster of patients. In those endemic regions where the forests are cleared Russian spring-summer encephalitis is rare because of the scarcity of infected ticks and rodents. It should be noted also that not all virgin forested areas are reservoirs of this disease.

The overall morbidity and mortality rates for Russian spring-summer encephalitis are not accessible to the author. However, various reports are avail-

TABLE 5

*The incidence of Russian spring-summer encephalitis among non-vaccinated inhabitants of certain endemic districts*

YEAR	DISTRICT	POPULATION	CASES	REMARKS
1932-1941	56-66th, 64, 70 B, 12 B	?	50-60	Cases per year
1939	12 B	523	13	Refers to one collective
1940	12 B	51	3	Refers to one collective
1939	Khabarovsk	1,185	26	
1939	{ Maritime	4,900	33	
1940	{ Krai	6,980	38	

Compiled from Pavlovskii, E. N.: *Rôle of the Parasitologic Factor in the Epidemiology of Spring-Summer Encephalitis*, Moscow, 1940.

Kheifets, B. I.: *Sovetskaia Med.*, 1943, 10: 11.

able covering outbreaks in certain regions and collectives (30, 32, 33) and from these one may arrive at reasonably valid incidence figures for the endemic areas (see table 5).

The mortality rate ranges from 25% to 30% (4), the lower rate prevailing in children (34). In adults over 50 years of age the rate is not elevated as is the case in Japanese B encephalitis.

A striking feature of the disease is its well demarcated seasonal occurrence. Cases begin to appear in late April, reach the epidemic peak in late May or early June, and subside in late July. Thus, while there is some overlapping in the seasonal occurrence of Russian spring-summer and Japanese B encephalitis, they occupy different seasonal segments of the year.

The preponderance of cases is among males because the occupations most liable to afford contact with the infected ticks are physically demanding and more suited to their sex. Contact and familial cases are rare.

In summary, the available data on Russian spring-summer encephalitis show it to be a serious infection limited to certain small and well demarcated virgin

forested foci. It is intimately associated with those occupations in which there is exposure to infected, blood-sucking, ixodid ticks. At present there is no evidence that the disease is being spread to new areas.

*B. Vectors:* Russian spring-summer encephalitis is the only known viral disease naturally occurring in man, in which the bite of an infected tick is the main mode of transmission. As a result of a series of yearly expeditions sent into the forests and collectives of eastern Siberia to study the health and disease hazards of this area, the encephalitis of the forested regions was particularly investigated and the role of the tick vector thoroughly explored.

The epidemiology of the disease, together with the rarity of contact and familial cases, led to the postulation of an arthropod vector (30). The pasture tick, *Ixodes persulcatus*, was known to occur regularly in the endemic foci and to bite man with avidity. Laboratory studies showed that *I. persulcatus* could be infected readily when allowed to feed on mice infected with the spring-summer virus. In mice and in other rodent hosts inhabiting endemic areas, the virus is present in the circulation (35). By the sixth day after feeding, virus was found to be present in the salivary glands (35) and to persist there for long periods of time (36). A prolonged period of feeding, approximately 48 hours, is necessary before the infected tick can transmit the disease. When infected ticks were allowed to feed for only two hours on healthy white mice, transmission of the disease did not occur (36). It has been reported also that the infected female *I. persulcatus*, as well as the female *Dermacentor silvarum* and *Haemaphysalis concinna*, lay infected ova which develop into adults harboring the virus (36).

While it was being established that ixodid ticks could be infected experimentally, these potential vectors were being examined in areas where Russian spring-summer encephalitis was prevalent. These studies indicated that *I. persulcatus*, *H. concinna*, *D. silvarum* and probably *H. japonica* were all naturally infected, and that *I. persulcatus* was the major vector.

The following figures give an approximation of the number of infected ticks occurring in endemic foci. Nineteen hundred *I. persulcatus* were collected from April 22nd to May 27th. "From this lot spring-summer virus was isolated nine times" (37). In another experiment, out of 1917 ticks of the ixodid family inoculated into mice twenty-eight were found to be infected (36). The tick strains and human strains of virus are apparently identical.

The principal hosts upon which these arthropods feed are the smaller forest rodents which in turn comprise the other half of the natural transmission cycle. The presence of neutralizing antibodies to the spring-summer virus in both naturally and experimentally infected rodents has been adequately demonstrated. The principal animals found infected in regions where the disease is prevalent are the chipmunk, the squirrel, the field mouse, the coriak rat, the hare, the hamster, the porcupine and the mole (38). In these hosts the disease may be symptomless.

*C. Clinical description:* Extensive descriptions of the clinical aspects of this disease are recorded in Russian by Panov (32), Pavlovskii, Krol and Smorodintseff (39), Altschuller (40), Glasunov (41), Robinson and Sergeeva (42).

About 75% of all cases give a history of a tick bite eight to eighteen days prior to the onset of symptoms (30). Following this incubation period, the onset of disease is abrupt. The patient has severe headache, as well as pain and tenderness in the cervical region. The temperature is elevated to 102 to 104 degrees Fahrenheit. Nausea, vomiting and some degree of vertigo are frequently present. It is claimed (32) that there is usually less delirium encountered in Russian spring-summer encephalitis than in Japanese B encephalitis. Coma is common. Meningeal inflammation and focal lesions in the central nervous system develop rapidly, resulting in paralysis of the muscles of the limbs, neck or back. Russian spring-summer encephalitis characteristically involves that portion of the central nervous system innervating the cervical and brachial musculature and results in paralysis of the shoulder girdle. The duration of acute symptoms is from two to ten days with a five to six day average. Paralysis most commonly occurs on the second or third day of the disease. Fatal cases result usually from involvement of the respiratory centers of the medulla. In such bulbar types it is difficult to rule out poliomyelitis, as disturbances of respiration, swallowing and phonation are common to both. Fatal cases expire with great rapidity, usually between the third and eighth day after the onset (32). The mortality rate is about 30% in non-vaccinated individuals.

Residuals in Russian spring-summer encephalitis occur in approximately 20% of the cases, the most common being paralysis and atrophy of the muscles of the neck and shoulder girdle. In endemic areas many of the local inhabitants exhibit a shoulder drop or torticollis as a result of a previous encounter with this infection. However, Parkinsonian phenomena seldom follow an attack of this disease.

*D. Pathology:* The following description is based on Smorodintseff's report (30).

The main feature of the pathology of Russian spring-summer encephalitis is severe inflammatory and degenerative changes in the brain, cord, sympathetic nervous system and peripheral nerves. Kestner (43) has described the process as an "acute, non-suppurative meningo-polioencephalomyelitis."

On gross examination the meninges have an appearance similar to that found in acute serous meningitis. The brain is congested with numerous petechial hemorrhages in the brain stem and medulla. The regions of greatest involvement are the gray matter of the base of the brain, medulla and cord. In the frontal and parietal cortex the changes are relatively slight.

Microscopically, there is an acute perivascular infiltration of the blood vessels of the entire brain with a diffuse or focal round cell accumulation in the gray matter.

Of greatest importance are the degenerative changes undergone by the neurones of the medulla and the spinal cord. All stages of cellular degeneration are noted from early chromatolysis and loss of Nissl substance to complete necrosis and neuronophagia. It should be observed that extensive neuronophagia is rarely described in the pathology of Japanese B encephalitis.

Small focal hemorrhages in the gray matter are frequently observed and may

vary in number and size depending upon the case. The cellular infiltrates are composed mainly of lymphocytes and a small number of polymorphonuclear leukocytes and glial cells.

*E. Laboratory diagnosis:* There are no changes in the chemistry or leukocyte counts of blood or spinal fluid which are pathognomonic of Russian spring-summer encephalitis. The spinal fluid cell count is usually between 25 and 100 cells. Lymphocytic cells predominate.

The virus of Russian spring-summer encephalitis is present in the circulation of man during the acute phase of the disease and has also been isolated from the spinal fluid at this time. For this reason the inoculation of susceptible laboratory animals, e.g., mice, hamsters or monkeys with whole blood taken as soon as possible after the onset of symptoms offers a method of making a laboratory diagnosis. It has been observed that whereas most of the encephalitic viruses are not highly infectious when administered intraperitoneally to adult white mice, the agent of Russian spring-summer encephalitis, once it has been established in mice, will regularly kill such animals when injected intraperitoneally even when diluted to one part in ten million. This characteristic offers a rapid method for differentiating the Russian spring-summer virus from that of Japanese B, both of which may occur in the same region.

The serological differentiation of this virus from those causing other acute viral encephalitides requires the employment of suitable techniques such as the complement fixation or neutralization test. According to Smorodintseff, neutralizing antibodies appear about one month after onset and persist for many years.

*F. Vaccination and serotherapy:* As soon as the Russian investigators had established the etiology of spring-summer encephalitis, a program was instituted, particularly at the Institute of Epidemiology and Microbiology in Moscow, for the development of a suitable vaccine. Such a vaccine was prepared and descriptive reports of it have appeared by Smorodintseff, Levkovich, and Dankovskii (44), Kagan (45), etc.

The vaccine in use at present is prepared from infected mouse brain in which the virus has been inactivated with formaldehyde. To a 0.5 to 1% emulsion of infected tissue, formalin is added to a final concentration of 1:500 to 1:750. After storage in the refrigerator for from ten to fifteen days to permit detoxification the vaccine is ready for use. Such a preparation is stated to retain its antigenicity for sixty days or "longer" (30). Vaccine is administered subcutaneously in two doses, a first inoculation of 3 cubic centimeters is followed by a second dose of 5 cubic centimeters, six to ten days later. There is no mention of an untoward reaction in many thousands who have received this rather heroic prophylaxis.

The efficacy of the mouse brain vaccine is apparently well established. The following table (table 6), the data for which was collected from various sources (30, 46), shows the morbidity and mortality among selected groups of vaccinated and non-vaccinated humans residing in the same region.

The employment of convalescent human serum inoculated intrathecally has been advocated as a therapeutic measure. This is interesting in view of the almost invariably negative results following this type of therapy in the other virus encephalitides.

IV. THE SEROLOGICAL RELATIONSHIPS BETWEEN THE AGENTS OF JAPANESE B, RUSSIAN SPRING-SUMMER, AND OTHER KNOWN VIRUSES CAUSING ENCEPHALITIS

Within the past three years workers in the field of the neurotropic viruses have been engaged in studies dealing with the serological relationships which exist between the above-mentioned strains and certain other viruses causing acute encephalitis. Foremost among the latter are the St. Louis encephalitis and the louping ill viruses.

The St. Louis strain was isolated in 1933 from the epidemic of acute encephalitis which occurred in that city. Serologically, this agent has been shown to have slight but definite relationship with Japanese B (47). However, cross immunity between these two infections is probably non-existent in the light of animal experiments. It will suffice for the purposes of this review to state

TABLE 6

*The effectiveness of vaccination with the mouse brain vaccine for Russian spring-summer encephalitis*

TREATMENT	NUMBER	CASES	DEATHS	REGION AND OCCUPATIONS INVOLVED
Non-vaccinated.....	2,942	44	11	None given
Vaccinated.....	1,527	2	0	
Non-vaccinated.....	1,185	26	7	Khabarovsk, 1939, lumbering collective
Vaccinated.....	985	2	0	
Non-vaccinated.....	51	3	2	Khabarovsk, 1940, lumbering collective
Vaccinated.....	1,996	0	0	
Non-vaccinated.....	6,980	38	9	Maritime Krai, 1940, lumberman
Vaccinated.....	4,900	0	0	
Non-vaccinated.....	10	7	2	Birobidjan, 1939, lumberman
Vaccinated.....	220	0	0	

that the virus of Japanese B encephalitis is related to, but readily distinguished from the St. Louis strain. There is no cross reaction whatsoever between Russian spring-summer encephalitis and St. Louis encephalitis.

In contrast, the virus of louping ill was shown in 1943 by Casals and Webster (48) to be closely related and perhaps identical with the virus of Russian spring-summer encephalitis. Louping ill is a disease of sheep which has been endemic in Scotland and northern England for over a century. The disease takes the form of an acute encephalomyelitis. The sheep tick, *Ixodes ricinus*, is the vector of this agent and although the disease has occurred in laboratory workers no records exist of naturally occurring louping ill in man. We thus have a disease restricted to Scotland and northern England, limited in nature to sheep and not man, and a disease apparently occurring only in the U. S. S. R. frequently attacking man in which the causative agents are probably the same.

Louping ill and Russian spring-summer encephalitis are not related to St.

Louis encephalitis but, as previously mentioned, there exists some uncertainty as to whether a small degree of common antigenicity exists between the Russian spring-summer and Japanese B viruses as some cross neutralization is claimed by certain Russian workers (30).

The reasons for the uncertainty as to whether the same virus causes both Japanese B and Russian autumn encephalitis are worth mentioning. As has been pointed out previously, both diseases have a similar epidemiology and clinical course. Furthermore, on the basis of certain Russian protocols, convalescent sera from cases of autumn encephalitis cross-neutralize the Japanese B virus to a degree equal to that obtainable with Japanese B immune sera. In a similar fashion Japanese B immune sera protect against the autumn strains. Cross immunity between the two viruses in mice is also said to be demonstrable. Furthermore, Levkovich (49) states that antisera against the Russian spring-summer virus act upon both the autumn and the Japanese B strains, whereas sera against the latter two viruses do not neutralize the spring-summer agent.

In American studies (50, 51) on the other hand, no such serological relationship between the Russian spring-summer and the Japanese B virus (Nakayama strain) could be demonstrated. In addition, a strain of virus sent from Moscow to the United States and labelled "Fall, Japanese encephalitis, strain Zagan" proved to be identical with the spring-summer virus and was serologically unrelated to Japanese B. Further experimentation with other specimens of virus from cases of "autumn" encephalitis is necessary to clarify the situation.

#### V. OTHER EPIDEMIC ENCEPHALITIDES OF THE FAR EAST

Encephalitis lethargica (Type A encephalitis) has been reported throughout the Far East. The causative agent and mode of transmission are unknown although a number of agents have been suggested but not proved. Epidemiological reports are often unsatisfactory inasmuch as one cannot be sure that all descriptions of "lethargic encephalitis" in the Far East refer to the same entity. The disease occurs during the winter months and the presence of residual especially of the Parkinsonian type are frequent. Both of these phenomena are rare in Japanese B and Russian spring-summer encephalitis.

Kaneko and Aoki (1) in a review of the reported cases of encephalitis of all types in Japan up to 1928, listed 223 cases of encephalitis lethargica occurring mostly in the winter months and never in epidemic form as is the case with Japanese B encephalitis. The disease has not been reported from Formosa and is rarely encountered in the Philippines. Kuttner and Tung (9) stated (1936) that encephalitis of any kind is rare in China. Watson (52) and Yew and Watson (53) noted that Yunnan Province has an incidence of only 15 to 20 cases of all types of encephalitis each year. There are no available figures to indicate the frequency of the lethargic type in Korea, Manchuria and eastern Siberia.

In summary, it seems reasonable to believe that at present this disease does not constitute a grave problem in any of these areas, nor does it exhibit any greater frequency in those regions in which either Japanese B encephalitis or Russian spring-summer encephalitis are endemic.

TABLE 7  
*Some characteristics of the encephalitides of medical importance in the Far East*

TYPE OF ENCEPHALITIS	SEASONAL OCCURRENCE	ENDEMIC AREAS KNOWN AT PRESENT	CAUSATIVE AGENT	VECTOR	RESERVOIR KNOWN AT PRESENT	FATALITY RATE	VIRAL RELATIONSHIP
Russian spring-summer	April-June	Northern Russia Siberia Maritime Region	Virus	Ticks	Ticks Rodents	30%	Related closely to louping ill. Possibly to Japanese B.
Japanese B	July-Sept.	Japan China Korea Maritime Region	Virus	Mosquitoes	Mosquitoes Rodents (?) Birds (?)	60%	Probably same as Russian autumn encephalitis.
Russian autumn (?)	Aug.-Sept.	Siberia Maritime Region	Virus	Mosquitoes	Mosquitoes Rodents (?) Birds (?)	50-60%	Probably identical with Japanese B encephalitis.
Encephalitis lethargica (von Economo)	Mainly winter months	No recent epidemics reported	Unknown	Unknown	Unknown	35-50%	
Encephalitides not reported from the Far East, but related to certain of the Asiatic types							
St. Louis	Summer-autumn	Western & Central United States	Virus	Mosquitoes	Mosquitoes & mites (?) Rodents (?) Birds (?)	20-30%	Distantly related to Japanese B on basis of serological reactions.
Louping ill (in sheep)	Spring and some in autumn	Scotland England	Virus	Ticks	Ticks	Does not naturally involve man	Closely related to & perhaps identical to Russian spring-summer.

Equine encephalomyelitis has never been reported as epidemic in man in Asia.

Table 7 summarizes some of the more pertinent epidemiological and immunological data of the encephalitides of the Far East.

#### VI. COMMENT

The reader of this review will perhaps appreciate the many problems still to be solved concerning the etiology and control of Japanese B and Russian spring-summer encephalitis. For instance, neutralizing antibodies to the Japanese B virus have been found in American students who had never lived in an area where the disease occurred (29). What is the significance of these antibodies? To what extent are the Chinese, particularly in rural areas, afflicted with mosquito-borne encephalitis? What constitutes the important reservoirs of Japanese B encephalitis? Can arthropods, other than the Ixodidae, be infected with and naturally transmit the virus of Russian spring-summer encephalitis?

The problem of effective vaccines for the control of these diseases is being actively investigated here and abroad. It is hoped that by means of such vaccines together with an effective insect control program utilizing the newer insecticidal compounds and aerosols it will be possible to prevent these encephalitides from reaching epidemic proportions.

#### ADDENDUM

Since the submission of this article several reports have appeared concerning these diseases.

In the first week of July 1945 an outbreak of Japanese encephalitis occurred among the natives and some of the U. S. military personnel on the island of Okinawa (Hodes, H. L., Thomas, L., and Peck, J. L., *Proc. Soc. Exp. Biol. & Med.*, 1945, **60**, 220). Prompt diagnosis of the disease was accomplished by means of complement fixation tests with the sera from clinical cases and with antigen prepared from a virus isolated from the brain of a fatal case. The reports of the clinical, epidemiological and vaccination studies will undoubtedly appear shortly from a number of sources.

A Japanese encephalitis vaccine prepared from infected chick embryo tissue has been described (Warren, J. and Hough, R. G., *Proc. Soc. Exp. Biol. & Med.*, 1946, **61**, 109). The preparation has immunogenic properties in mice and men comparable to mouse brain vaccines.

The contradictory statement in the literature (see Levkovich, reference #49, and Casals, reference #50 above) regarding the relationship between strains of Russian spring-summer encephalitis and Japanese encephalitis have apparently been resolved. Dr. A. A. Smorodintseff, Academy of Medical Sciences, U.S.S.R., in a communication to Lt. Colonel Joseph E. Smadel, MC, November 1945, makes the following statement:

"Conclusions made by Dr. Casals . . . concerning the antigenic structure of Russian encephalitis are completely confirmed in my laboratory during the past months. Our spring-summer encephalitis is not related to the Japanese B nor Russian autumn encephalitis. With the new strains of Japanese B viruses,



brought from U.S.A., we have been unable to establish any immunological relationship with our strains of spring-summer encephalitis. The work, reported by Levkovich, was made with a "Kalinin" strain, received in 1937 from Japan. This strain was probably contaminated by spring-summer virus or was not typical for Japanese B group of viruses. Now this strain is lost. On the other hand it appears obvious that our autumn and Japanese B viruses are immunologically identical and showed a close relationship by complement fixation, neutralization and cross immunity tests. Strain "Zagan" does not belong to the autumn encephalitis group and was sent to U. S. by mistake. . . ."

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# EXPERIMENTS WITH VARIOUS COLOMBIAN MARSUPIALS AND PRIMATES IN LABORATORY CYCLES OF YELLOW FEVER\*

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Most recent work on the susceptibility of various mammals to the virus of yellow fever has been carried out by the method of inoculating the animals with calculated doses of virus and then bleeding them at regular intervals to test for virus circulation. By this method it is possible to test large numbers of individuals and thus gain an idea of the relative susceptibility of different species and of different mammal groups, as was done for instance for marsupials by Bugher and his co-workers (1941). Most mammals that have been tested by this means, however, undergo inapparent infections with relatively small amounts of virus in circulation in the blood stream. The rather extensive field studies of sylvatic yellow fever that have been carried out in Brazil and Colombia all indicate that the virus is maintained in nature by constant mammal-mosquito passage, and that in many areas at least the vector mosquito is *Haemagogus capricornii* or closely related species (Bugher *et al.*, 1944).<sup>1</sup> In the laboratory this mosquito requires rather special conditions for infection, and an important condition is the amount of virus in circulation in the host mammal (Bates and Roca, 1945a). It would thus seem to be necessary, if laboratory results are to be interpreted in epidemiological terms, to make susceptibility tests of a given mammal species using infected mosquitoes as the inoculating agent, and to attempt to infect new lots of mosquitoes with this mammal as host.

It would also seem advisable to make such transmission experiments, as far as possible, with local materials. *Haemagogus* mosquitoes apparently differ from *Aedes aegypti* in infection behavior (Antunes and Whitman, 1937); and it seems quite possible that the various other vector mosquitoes may differ considerably among themselves in this regard, so that generalization from one species to another would be hazardous. The use of local virus strains seems also to be important. Laemmert (1944), for instance, has shown how much virus strains may differ in their behavior in marmosets, and we have found that laboratory manipulation (especially mouse brain passage) may considerably modify the properties of a virus strain both in mammal and mosquito hosts. There is even a certain amount of evidence that there may be geographical variation in the susceptibility of a given mammal host species—the most clear-cut instance

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<sup>1</sup> This population is identified as a form of *Haemagogus spegazzinii* in a recent paper by Kumm, Osorno, and Boshell (1946).

perhaps being that of the Nigerian and Sudanese hedgehogs (Findlay and Mahaffy, 1936). In other words, the acid test of a possible transmission mechanism is laboratory reproduction with local mammals, local mosquitoes, and local virus.

We at one time planned to make an extensive series of tests of this sort with mammals and mosquitoes found in the Villavicencio area, but a change in laboratory program has involved a suspension of yellow fever transmission studies. Experiments of this sort with saimiri and douroucoulis monkeys have already been described (Bates and Roca, 1945a and 1945b), and it may be worthwhile to publish results that we have obtained with a few other mammal species, even though the experiments are not as complete as we would like to have them. They may serve to show the possibilities of the technique in working with mammals of questionable epidemiological importance.

The methods and materials used in these studies have been fully described in a previous article (Bates and Roca, 1945a). We are greatly indebted to Dr. G.H.H. Tate of the American Museum of Natural History for the identification of our mammals and for his thoughtful comment on the relationships of various forms.

#### MARSUPIALS

The discovery by Bugher *et al.* (1941) of the susceptibility of various species of marsupials to laboratory infection with yellow fever virus aroused considerable interest in the possible rôle of these animals in the maintenance of virus in nature. The species sufficiently common to be of interest in this connection belong to five genera, *Didelphis*, *Metachirops*, *Metachirus*, *Caluromys*, and *Marmosa*, all except the last with only one species each in eastern Colombia. Specimens of *Didelphis* and *Metachirops* only rarely show circulating virus after subcutaneous inoculation, and when virus can be demonstrated in the blood stream, the titer in circulation is very low (Bugher *et al.*, 1941). *Marmosa* is a protean genus with many species, difficult for the non-specialist to classify. The single specimen studied by Bugher *et al.* (species unidentified) proved to be susceptible; the few tests that we have made with other local specimens have given negative results. From unpublished studies that we have seen, it seems likely that susceptibility to the virus varies greatly among species of the genus; a study of the possible relation of these animals to virus cycles would thus have to be a special and somewhat complicated study. *Metachirus* (the brown masked opossum) and *Caluromys* (the woolly opossum) have both proved in the laboratory to be susceptible to infection with a wide variety of strains of yellow fever virus (Bates, 1944b and unpublished studies by C. R. Anderson and Roca), a majority of individuals showing peripheral circulation of virus, sometimes in considerable amount. *Metachirus* shows the more regular infection behavior of the two, and in consequence it was selected for study in connection with the transmission cycles. We felt that if *Metachirus* could not be interposed in the cycles, no other marsupial could. Notes on the identification of *Metachirus* and *Caluromys* have been given in a previous article (Bates, 1944b).

TABLE 1

*Historics of Metachirus bitten by infected Haemagogus*

METACHIRUS NO.	HISTORY OF MOSQUITOES			CIRCULATING VIRUS: DAY					MOSQUITO FEEDINGS (LOT NOS.)
	Lot No.	No. biting	Days* infection	3	4	5	6	7	
OA228	140	5	17	+	+	-	-	-	
OA226	142	3	17	-	-	-	-	-	
OA229	190	3	20	+	-	-	-		201 and 202
OA230	191	5	19	+	+	†			200
OA231	190	1	27	+	+	+	-	-	206
OA232	191	4	26	+	+	-	-	-	207 and 205
OA233	195	4	13	+	+	+	-	-	
OA234	206	4	25	+	+	-	-	-	
OA237	233	2	24	-	-	+	-		242
OA238	233	2	24	+	-	-	-		241 and 243
OA239	233	2	32	+	-	-			248
OA240	233	2	32	-	-	†			
OA241	240	3	22	+	+	†			251

\* All mosquitoes kept at a constant temperature of 30°C.

† Death from extraneous cause.

TABLE 2

*Attempts to infect haemagogus mosquitoes on Metachirus*

HAEMAGOGUS LOT NO.	METACHIRUS		ADULT MOUSE MORTALITIES		ATTEMPTS AT VIRUS RECOVERY	
	No.	Day of infection	serum dil. 1:10	control mos- quitoes	By mosquito bite	By inoculation of mosquitoes
200	230	3	3/5	3/10	Neg. at 16 days to saimiri	Saimiri infected by pool of 3 at 16 days
201	229	3	6/6	3/10	None	None
202	229	4	0/6	0/11	None	None
205	232	3	5/5	1/16	None	1 at 26 days negative in mice
206	231	4	6/6	15/16	Transmitted at 14 and 17 days to saimiris; at 25 days to Metachirus	None
207	232	4	6/6	4/13	Neg. at 22 days, saimiri	4 at 22 days negative in mice
241	238	3	6/6	3/10	Neg. at 15, 20, 23 and 26 days	1 of 10 mosquitoes positive on mouse inoculated
242	237	4	0/6	0/18	None	None
243	238	4	0/6	0/17	None	None
248	239	4	0/4	0/16	None	None
251	241	3	5/5	2/18	None	2 at 25 days negative in mice

*Metachirus nudicaudatus*

The histories of 13 *Metachirus* that were bitten by infected haemagogus are summarized in table 1. Eleven of the 13 showed circulating virus on one or more days. The 2 animals that failed to show virus were involved in experiments in which we cannot be sure that the individual mosquitoes used for biting contained virus, though they formed part of lots that transmitted to other animals and that should have been infective. The percentage of *Metachirus* showing virus in these experiments (85 per cent) is higher than the average for animals infected by *in vitro* inoculation (72 per cent, Bates, 1944b). It is interesting that virus was

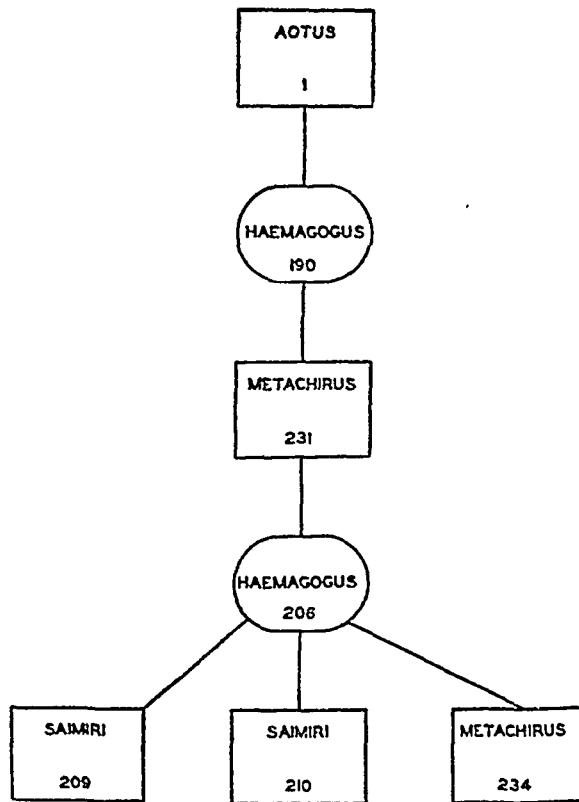


DIAGRAM I. CYCLIC PASSAGES WITH BROWN MASKED OPOSSUM

most often circulated on the 3rd day, while after *in vitro* inoculation it was most often found on the 4th day; from this and from observation of monkey infections, we judge that the effective dosage inoculated by mosquitoes is high. Largely for this reason, we have generally allowed only a very few infected mosquitoes to bite a given animal.

Eleven attempts to infect haemagogus on *Metachirus* are summarized in table 2. In 4 cases it was found that the animal had no virus in circulation at the time of the attempted infection. In 6 cases there was a small amount of virus in circulation, but not enough to kill more than half of the mice inoculated with suspensions of control mosquitoes. In only 1 case (mosquito lot 206) was there a large

amount of virus in circulation at the time of feeding. Titrations were not made on the sera of these marsupials, since the uncertainty of the results seemed hardly to warrant the large expenditure of mice that would be involved. This particular *Metachirus* (No. 231) on the 4th day must, however, have been circulating a high titer of virus, since 15 of 16 mice inoculated with control mosquito suspensions were killed; this is comparable with the control mortalities of successful haemagogus infections on saimiri and douroucoulis monkeys. These mosquitoes transmitted virus to a *Saimiri* at 14 days (6 biting) and at 17 days (again 6 biting), and to a *Metachirus* at 25 days (4 biting). The sequence of these passages is shown in Diagram No. I.

The infection of a saimiri monkey by the inoculation of a pool of 3 mosquitoes of lot 200, and the recovery of virus from 1 of 10 mosquitoes of lot 241 tested by intracerebral inoculation in mice, shows that it is occasionally possible to infect haemagogus on *Metachirus* circulating small or moderate amounts of virus. The transmissions by the mosquitoes of lot 206 show that *Metachirus* can at times be interposed in laboratory cycles. The difficulties experienced with this species, however, contrast strongly with the ease with which mosquito infection cycles can be maintained with truly susceptible mammals such as the primates *Saimiri*, *Aotus*, and *Oedipomidas*. Among other things, it is difficult to induce haemagogus to feed on these marsupials. It takes as much time and effort to induce 20 mosquitoes to feed on a *Metachirus* as it would to get 100 to feed on a monkey.

#### *Caluromys laniger*

Four woolly opossums were fed on by mosquitoes in the series of cycles of Rodas virus, under circumstances that should surely have resulted in infection. Only 1 of these showed circulating virus (bitten by 4 mosquitoes of lot 195, 19 days after infection on *Aotus* 2). Virus was recovered from this animal on the 3rd, 4th, 5th, 6th, and 7th days, and on the 4th day the titer may have been high since all 6 mice inoculated with the 1:10 serum dilution were killed. It would probably be possible to interpose an occasional animal of this species in the cycles, as was done with *Metachirus*.

#### PRIMATES

Primates have been of special interest in relation to yellow fever since the discovery by Stokes, Bauer, and Hudson (1928) of the susceptibility of the rhesus monkey. Studies of the susceptibility of a wide range of South American primates were carried out by N. C. Davis, and the results were published in a series of papers (Davis, 1930a, b, c; 1931; Davis and Shannon, 1929). These studies were carried out before the white mouse was available as a laboratory tool for determining amounts of virus in circulation, and most of the tests were made with African virus (Asibi strain) and with *Aedes aegypti* mosquitoes. It is consequently not always possible to determine the degree of susceptibility shown by the various species. Davis's work, however, demonstrated that South American monkeys as a group were very generally somewhat susceptible to infection with the virus of yellow fever in that virus could be recovered in circulation, though

most species showed no clinical symptoms of infection. He found that the marmosets *Callithrix* and *Leontocebus* and the cebid monkey *Saimiri* were more susceptible than the others, infection frequently being fatal. None of the South American monkeys, however, showed the uniform infection behavior and clear diagnostic hepatic lesions of the Indian rhesus; they consequently seemed unsuitable as laboratory animals. Recently Laemmert (1944) re-examined the susceptibility of marmosets, and Bates (1944a) that of *saimiri* monkeys, and these animals are now used routinely in yellow fever studies in South American laboratories.

The study of South American monkeys is greatly handicapped by the confused state of primate nomenclature. This is partly due to the uncertain status of many old names, and partly to the difficulty in interpreting the extensive geographical variation shown by most of the groups. The result is that a non-specialist has no chance of being able to identify a given specimen, and little chance of being able to interpret the various identifications published in the literature. Since the American primates are of great interest from many points of view, this nomenclatorial confusion seems particularly unfortunate. The spider monkeys (*Ateles*) are the only group to be reviewed recently (Kellogg and Goldman, 1944). The popular book by Hooton (1942) is an excellent introduction to the general literature on monkeys, and Ruch (1941) has published a detailed bibliography on the anatomy and physiology of primates. The paper by Tate (1939) contains extended notes on the monkeys of northern South America.

The South American monkeys as a group are distinct from all of the Old World primates. They are in turn generally divided into 2 families, the more primitive Callitrichidae or marmosets, and the Cebidae, including all of the other South American monkeys. The vernacular nomenclature of these animals is about as confused as the scientific nomenclature. Simpson (1941) has recently made a laudable attempt to introduce order into the vernacular names, but we do not agree with all of his proposals. He would limit the word "marmoset" to the genus *Callithrix*; but the word is very commonly and appropriately used for all of the members of the family. The various types of marmosets seem not to have acquired distinctive vernacular names. "Titi," which Simpson would apply to *Callicebus*, is used in various countries for so many different monkeys that it seems better to avoid the word entirely.

#### *Oedipomidas oedipus*

This is the common marmoset of central Colombia (fig. 1). It apparently does not cross the eastern Andes, and to the best of our knowledge there are no marmosets at all in the region of Villavicencio. We became interested in the possible susceptibility of *Oedipomidas* to yellow fever because it seems, ecologically, to replace *Saimiri* in central and western Colombia. Similarly, in Panama, *Oedipomidas geoffroyi* is common about the Canal Zone and in eastern Panama, being replaced by *Saimiri orstedii* in western Panama (Goldman, 1920). *Saimiri* and *Oedipomidas* are both known in Panama and Colombia as "titis," though they are completely unrelated and do not look alike.



We secured 4 of these marmosets from a dealer, and interposed them in transmission cycles, as shown in the diagram accompanying the section on "maintenance of cycles" later in this article. Virus was carried from *Aotus* to *Oedipomidas*, *Oedipomidas* to *Oedipomidas*, and *Oedipomidas* to *Aotus* and *Saimiri* through the bite of *haemagogus* mosquitoes, without the slightest difficulty. It will be noted that the 4 *Oedipomidas* were infected in series; the first 3 showed fatal infections, dying on the 7th, 9th, and 6th days respectively, while the 4th animal survived the infection. The animals all showed virus in circulation from the 3rd through the 7th day (not tested earlier or later), with very high titers. We did not reach the end point with either of the first 2 animals, though dilutions were carried to  $1:10^5$ ; serum of the 3rd animal diluted  $1:10^7$  killed adult mice on both the 4th and 5th days (giving respectively 2/6 and 1/5 mortality ratios). More



FIG. 1. OEDIPOMIDAS OEDIPUS

complete titrations were carried out on the 4th animal (which showed a much milder, non-fatal, infection); the final dilutions killing adult mice were 3rd day,  $1:10^5$ ; 4th day,  $1:10^5$ ; 5th day,  $1:10^4$ ; 6th day,  $1:10^5$ ; 7th day,  $1:10^5$ . The virus titers of the first 3 animals seemed comparable to the very high titers shown by douroucoulis (Bates and Roca, 1945b). These marmosets, however, had no febrile reaction, and the post-mortem liver pathology was not characteristic of yellow fever, showing only a diffuse necrosis.

The reaction of these marmosets to yellow fever infection seems to be very similar to that shown by the Brazilian species of *Callithrix* and *Leontideus* reported by Laemmert (1944), and to that of the cebid monkey *Saimiri*, except that *Saimiri* fairly often has a definite febrile reaction (Bates, 1944a). It may be noted that these 3 genera of marmosets—*Callithrix*, *Oedipomidas*, and *Leontideus*—

represent the three main morphological divisions of the family (Tate, 1939); one wonders whether this means that all of the marmosets are highly susceptible to infection with the virus of yellow fever.

*Callicebus ornatus*

This is a common monkey in the Villavicencio area and like *Saimiri*, it persists near cultivated areas quite close to the town (fig. 2). It is known locally as the "socay." In other places it is sometimes called the "viudita," and the translation "widow monkey," which is occasionally used, seems appropriate to its doleful aspect in captivity. We have had a great deal of difficulty in maintaining these monkeys in captivity, and we have consequently not been able to test many of them with virus. Davis (1931) inoculated 2 monkeys tentatively identified



FIG. 2. *CALLICEBUS ORNATUS*

as *Callicebus moloch*, and in 1 case was able to transfer virus from *Callicebus* to rhesus monkeys both directly and through *Aedes aegypti*. This *Callicebus* showed fever on the 4th and 5th days and died on the 7th day.

In our experiments, 4 *Callicebus* were bitten by infected mosquitoes. The data on these are summarized in table 3. Only 2 of the 4 animals showed circulating virus. Of the 2 that failed to circulate virus, No. 7 survived for a post-inoculation protection test, giving a clear positive in contrast to the preinoculation negative. No. 11 died on the 5th day after being bitten; the routine autopsy showed nothing of interest, and the report on the pathological examination of liver material was "focal necrosis." Unfortunately neither of the animals that circulated virus was used for attempts at mosquito infection. No. 19, however, may have circulated an appreciable amount of virus, since the 1:10 dilution of

serum gave 6/6 mouse mortalities on the 4th and 5th days; No. 24 circulated small amounts of virus on the 3rd, 4th and 5th days, and died of an unknown cause on the 13th day.

Two *Callicebus* were inoculated subcutaneously with about 20 mouse m.l.d. of Perez virus. One of these was bled daily for 10 days, and showed traces of virus on the 3rd, 4th, and 5th days. The other showed a trace of virus on the 4th day, and a titer of over 1:100 on the 5th day. It died on the 6th day, and Dr. Augusto Gast-Galvis reported that the liver tissue "shows a necrosis similar to that of yellow fever, although diffusely distributed; necrotized acidophilic cells are present, in some cases similar to Councilman bodies with fatty inclusions."

It is difficult to interpret the results with these 6 monkeys, especially in view of the high mortality rate shown by normal animals in captivity. The species

TABLE 3

*Histories of Callicebus ornatus and Cebus fatuellus bitten by infected haemagogus*

MONKEY NO.	HISTORY OF MOSQUITOES			CIRCULATING VIRUS: DAY					POSTINOCULATION PROTECTION TEST†
	Lot No.	No. biting	Days* inf.	3	4	5	6	7	
<i>Callicebus</i>									
7	136	2	28	—	—	—	—		Positive
11	142	10	17	—	—	—†			
19	195	6	15	+	+	++‡			
24	195	3	24	+	+	+	—		
<i>Cebus</i>									
3	136	4	24	—	—	—	—	—	Negative
5	140	4	28	—	+	+	+	—	
6	136	5	26	—	—	—	—	—	Positive
10	191	11	22	—	—	+	—	—	

\* All mosquitoes kept at a constant temperature of 30°C.

† Preinoculation protection test in all cases negative.

‡ Death from extraneous cause.

should probably be classed as "moderately susceptible," and it seems likely that the amount of virus circulated varies greatly among individuals. We have made intracerebral protection tests on the sera of 25 animals caught during 1944 in the vicinity of Restrepo, where virus was known to be present in both 1943 and 1944; 2 of these sera were clearly positive for yellow fever antibodies, and 1 gave "inconclusive" results. The species is thus at times infected in nature, and it may play a part in the maintenance of the virus. It seems likely, however, that its rôle in natural cycles would be subordinate.

### *Cebus fatuellus*

The monkeys of the genus *Cebus* (fig. 3) are generally known in English as "capuchins" because of the fancied resemblance of their hair to a Capuchin cowl. They are the brightest of the American monkeys, and everyone is familiar

with their antics as assistants for organ grinders. The common name in the Villavicencio area is "maicero," from their habit of raiding maize plantations. The genus *Cebus* is protean, and the various local populations have been baptized with an incredible number of Latin names. The name *fatuellus* was proposed by Linnaeus, with "America" as the locality of origin; it is now, however, generally restricted to the crested capuchin monkeys of the upper Magdalena Valley (Tate, 1939), with which the Villavicencio population seems to be identical.

Four cebus monkeys were bitten by infected mosquitoes, as shown in table 3. Virus was recovered in circulation from only 2 of these: in one there were traces of virus on the 4th, 5th, and 6th days, and in the other a trace on the 5th day only. Four lots of haemagogus fed on these monkeys, but in 3 cases no virus was recovered from the control mosquitoes, so the lots were discarded.



FIG. 3. *CEBUS FATUELLUS*

One of 3 control mosquitoes of lot 204, which fed on Cebus 10 on the 5th day, showed a trace of virus. This lot was kept for 20 days at 30°, at which time 11 surviving mosquitoes were ground together in 1.0 cc. of diluent, and the *total suspension* was inoculated subcutaneously into a saimiri monkey. This monkey showed no reaction, did not circulate virus, and postinoculation serum remained negative, from which we conclude that no virus whatsoever persisted in the mosquitoes. A fifth cebus monkey, which was inoculated intraperitoneally with a small virus dose, showed traces of circulating virus on the 5th and 6th days. In no case has a cebus monkey shown any febrile reaction to infection.

Davis (1930a) was able to maintain virus by serial transfer of blood in cebus monkeys, and by transmission with *Aedes aegypti* from *Macacus rhesus* to *Cebus* and back to *M. rhesus*. The identification of his *Cebus* is doubtful: in his article the species are called *Cebus flavus* and *variegatus*; in a correction note pasted in,

these names are changed to *Cebus albifrons* and *frontatus*. Most of his specimens were of the *variegatus-frontatus* type, which would be geographical representatives of our *fatuellus*. More recently, Waddell and Taylor (1945) have maintained virus cycles in the laboratory using *Cebus versutus* and *Aedes aegypti*. *Cebus versutus* is again probably a geographical representative of the *fatuellus* group of capuchins (Tate, in correspondence). This raises the interesting question of whether susceptibility to the virus of yellow fever varies with the geographical populations of these monkeys, since our Villavicencio tufted capuchins would seem to be less susceptible than the Brazilian capuchin populations—though it may be noted that the Brazilian animals do not circulate such high titers of virus as, for instance, *Saimiri*, *Aotus*, or the marmosets. Waddell and Taylor found that virus strains showed considerable variation in virulence for cebus monkeys, and it may well be that the difference between the Brazilian and Colombian results with experimental cebus infections is a property of the virus strains rather than of the monkey populations.

Cebus monkeys captured in the Villavicencio area frequently show positive yellow fever protection tests: 3 of 14 animals tested in 1944 were positive, and Bugher *et al.* (1944) report positive cebus sera in connection with their field studies. It seems to us that these natural cebus infections in the Villavicencio area may well represent a "dead-end" in natural virus cycles, since it seems hardly likely that haemagogus would regularly become infected on these monkeys. *Cebus* is commonly found in company with *Saimiri*, and it would be equally exposed to infections; the question is how frequently it may serve as a source for new mosquito infections.

#### *Saimiri sciureus caquetensis*

The behavior of this monkey (fig. 4) in laboratory infections of yellow fever has been described in previous papers (Bates, 1944a, Bates and Roca, 1945a). We have continued to find it to be a satisfactory experimental animal for the maintenance of virus cycles and for other routine purposes. At one time we made 10 serial passages of Rodas virus in these monkeys, starting with *Saimiri* 241 infected by mosquitoes of the 6th laboratory cycle (see Diagram II), with the object of obtaining data on behavior after *in vitro* inoculation with this strain. The animals were generally inoculated intramuscularly with 0.06 of a 1:100 dilution of the serum of the preceding animal taken on the 4th day. The animals were bled daily from the 3rd through the 7th day, and serial dilutions of serum were inoculated into adult white mice to test for virus circulation. Two animals were used for the second and third passages, otherwise one for each passage. The results of this passage experiment are summarized in table 4. Two of the 12 animals survived the infection; all, with one exception (No. 262), showed high titers of circulating virus with the highest titer generally on the 4th day. Of the 10 animals that died in the course of infection, 7 were found at autopsy to have signs of stomach haemorrhage; when present, this seems to be diagnostic of death from yellow fever in these animals. Doctor Augusto Gast-Galvis kindly made pathological examinations of liver tissue from all of the fatal cases; in no instance were the lesions characteristic of yellow fever.

*Aotus trivirgatus*

The behavior of this species in transmission cycles was described in a previous article (Bates and Roca, 1945b). Eight more animals have been used in cycles



FIG. 4. SAIMIRI SCIUREUS CAQUETENSIS

TABLE 4

*Histories of saimiris used in serial passage of Rodas virus*

(Dosages calculated in terms of mouse m.l.d.: titer of circulating virus indicated by final dilution of serum causing deaths in 42-day-old mice)

PAS- SAGE NO.	SAI- MIRI NO.	DOSAGE INOC.	CIRCULATING VIRUS: DAY					MAX. TEMP.	DAY OF DEATH	STOMACH HAEMOR- RHAGE
			3	4	5	6	7			
1	241	bitten	1:10 <sup>6</sup>	1:10 <sup>5</sup>	1:10 <sup>6</sup>	1:10 <sup>3</sup>	1:10 <sup>2</sup>	40.0°	8	—
2	248	2 x 10 <sup>6</sup>	>1:10 <sup>2</sup>	1:10 <sup>5</sup>				40.0°	4	+
2	250	2 x 10 <sup>6</sup>	>1:10 <sup>2</sup>	1:10 <sup>6</sup>	1:10 <sup>4</sup>			40.3°	5	+
3	252	600	1:10 <sup>5</sup>	1:10 <sup>4</sup>	1:10 <sup>5</sup>	1:10 <sup>2</sup>	0	40.7°	7	—
3	253	600	1:10 <sup>4</sup>	1:10 <sup>5</sup>	1:10 <sup>2</sup>	0	0	40.3°	8	
4	255	500	1:10 <sup>4</sup>	1:10 <sup>5</sup>	1:10 <sup>2</sup>	0	0	40.0°	8	+
5	260	100	0	1:10 <sup>2</sup>	1:10 <sup>5</sup>	1:10 <sup>6</sup>		40.0°	6	+
6	262	1000	1:10 <sup>3</sup>	1:10 <sup>2</sup>	1:10 <sup>2</sup>	0	0	40.0°	8	
7	216	10	1:10 <sup>6</sup>	>1:10 <sup>6</sup>	>1:10 <sup>6</sup>	>1:10 <sup>6</sup>		40.2°	7	+
8	222	2 x 10 <sup>5</sup>	1:10 <sup>6</sup>	1:10 <sup>5</sup>				39.2°	4	+
9	228	4000	>1:10 <sup>6</sup>					39.8°	4	—
10	267	?	1:10 <sup>5</sup>	1:10 <sup>6</sup>				39.0°	4	+

with Rodas virus; one of these (Aotus 5) survived infection; 6 of the other 7 died on the 5th, 6th, or 7th day after infection, and pathological examination of the liver showed lesions diagnostic of yellow fever similar to those found in man.

The remaining animal (*Aotus* 14) died on the 9th day and Dr. Gast found the liver to be "negative for yellow fever"; this animal had circulated virus from the 3rd through the 7th day, and traces of changed blood were found in the stomach at autopsy. Of a total of 12 *Aotus* used in these cycles, it thus seems that 10 certainly died of yellow fever and one possibly from some extraneous cause, while one survived.

#### DISCUSSION: MAINTENANCE OF CYCLES

Virus of the "Rodas" strain was maintained in the Villavicencio laboratory by constant mammal-haemagogus passage from October 10, 1944 (the date of the infection of *Saimiri* 176) to October 1, 1945, when yellow fever studies were stopped and all infected mosquitoes killed. During this period the "source" virus strain went through 14 complete mosquito-mammal cycles, as shown in Diagram No. II. The mosquitoes used for maintenance passage were generally incubated at a constant temperature of 30°C., so that development of virus in the mosquitoes was undoubtedly more rapid than it would have been in nature. However, no particular attempt was made to secure minimum cycles, and this laboratory experience can probably be used for drawing deductions concerning events in nature. Transmission in nature might be much slower than in the laboratory series, but it would certainly not be faster. One passage a month might well be the maximum natural rate. This means that "explosive epidemics," such as are sometimes found in areas peripheral to the regions where sylvatic yellow fever is endemic might require several months to build up to the point where they are discovered.

The virus is in the monkey for 7 or 8 days at most, and in the mosquito for life, so that spread may well be more a function of the mosquito than of the mammal host. With an average of 30 days between passages, one would hardly expect the spread, in any event, to be fast. It has often been pointed out that human infections seem to be incidental—and accidental—in the course of sylvatic yellow fever. The human infections sometimes show a pattern that seems to indicate rapid and extensive virus spread—but this may well be an artefact, since the human infections may lag months behind the actual epizootic spread of the virus.

Three species of monkey were used in the maintenance cycles, as is shown in the diagram. We thought, at times, that our strain was losing virulence through continual passage through the same mammal, and this feeling—as well as the accident of what species might be most readily available—led us to shift from *saimiri* monkeys to *douroucoulis* to marmoset. In actual fact, the virus seemed to be just as virulent for *saimiris* at the end of the series as at the beginning, and our impression of loss of virulence was probably false, based on the inevitable ups and downs of virus concentration in such a series of passages. We varied the mammals, however, in part deliberately, as we felt that a variety of hosts would make for more "natural" conditions. We feel that it is significant that none of the mammals that we tried, other than *saimiri* and *douroucoulis* monkeys and the marmoset, showed sufficiently regular infection behavior to be interposed

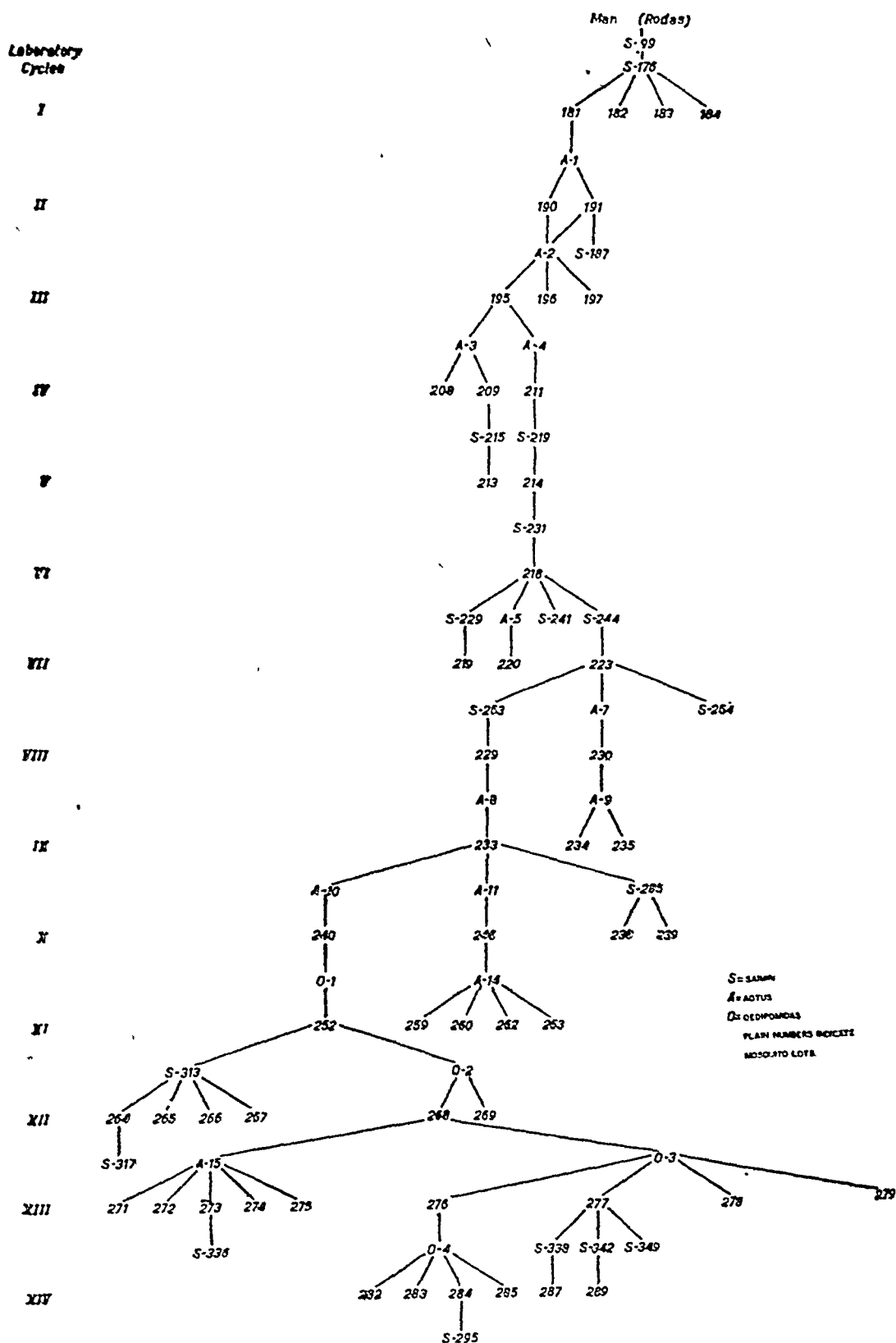


DIAGRAM II. MAINTENANCE PASSAGES WITH RODAS VIRUS



in the maintenance cycles. We had no difficulties whatsoever in maintaining virus with these three mammals, using haemagogus mosquitoes; and they surely would be equally efficient hosts in nature.

Only four groups of mammals would seem to be abundant enough in the South American forests to serve to maintain yellow fever virus: marsupials, rodents, bats, and primates. All experimental work with bats has given completely negative results (e.g., Kumm, 1932, and various unpublished studies in Brazil and Colombia). Apparently rodents vary greatly in susceptibility, and the group perhaps warrants further investigation. It has long been known that laboratory guinea pigs occasionally circulate virus (Sawyer and Frobisher, 1930), and in Villavicencio we have been able to passage virus serially by intraperitoneal inoculation of serum through up to 4 cane rats (the species discussed by Bates and Weir, 1944) before losing the virus, and circulating virus has occasionally been recovered from several other rodents. The habits of these rodents, however, do not coincide with those of haemagogus mosquitoes, and it seems very unlikely that any rodent, unless possibly some of the arboreal rats, would be associated with haemagogus infections.

The marsupials are another matter. We feel that species such as *Didelphis marsupialis* and *Metachirops opossum* can be ruled out, as far as haemagogus cycles are concerned, because they seem rarely if ever to circulate a sufficiently high titer of virus for haemagogus infections. *Metachirus nudicaudatus* obviously can at times form part of such cycles, since the mechanism has been reproduced in the laboratory. Recent field studies in areas of active yellow fever in Colombia, not yet published, show that individuals giving positive protection tests for yellow fever are rarely encountered among the more susceptible marsupials (*Metachirus* and *Caluromys*). Positive protection tests are more commonly encountered with *Didelphis* and *Metachirops* (Bugher et al., 1941, 1944) but it is possible that these reactions are in part, at least, non-specific (Bates, 1944b). Taking all of these factors into account, it seems to us rather unlikely that marsupials or rodents play any important part in the maintenance of natural mosquito cycles of virus. If some other type of arthropod, such as mites or ticks, were found to be capable of transmitting yellow fever virus, the whole problem of mammal hosts would of course have to be re-examined.

The American primates seem to be very generally susceptible in some degree to infection with the virus of yellow fever. The marmoset genera *Callithrix*, *Leontocebus*, and *Oedipomidas* and the Cebid genera *Saimiri* and *Aotus* all contain highly susceptible species, without any question capable of forming part of natural mosquito cycles. The recent recovery of virus from wild marmosets (Laemmert and Ferreira, 1945) is very significant in this regard. The howler monkeys (*Alouatta*) should probably be added to the list of highly susceptible groups, since there are various accounts of dead howler monkeys being found in connection with yellow fever epidemics. These monkeys, although often very common, are difficult to handle in the laboratory and almost nothing is known of their infection behavior; Davis (1931) tested one specimen, which showed a febrile reaction and served to infect *Aedes aegypti* mosquitoes. Some of the other Cebid genera seem to be of more doubtful importance with relation

to yellow fever, and we have been particularly impressed with our failure to get regular infections with the Villavicencio population of *Cebus fatuellus*. With the evidence at hand, it seems to us most likely that sylvatic yellow fever is maintained by constant mosquito-monkey cycles in nature; but it is unlikely that all species of monkey are equally important, and an evaluation of the situation in any given area would probably require the laboratory testing of all of the common local monkey populations.

#### SUMMARY

Attempts to interpose various marsupials and primates in laboratory cycles of yellow fever, using the mosquito *Haemagogus capricornii* as vector, are described. Of 11 attempts to infect haemagogus on the brown masked opossum (*Metachirus*) only one was successful, though 11 of 13 animals bitten by infected haemagogus showed circulating virus. One of 4 woolly opossums (*Caluromys*) showed circulating virus after being bitten by infected haemagogus. These two species are considered to be the most susceptible of Colombian marsupials, and it thus seems doubtful whether any of the local marsupials can play an important rôle in haemagogus cycles in nature.

The maintenance of cycles with haemagogus mosquitoes and various primates was relatively easy, and constant mosquito-mammal passages were maintained for a year (14 cycles) using saimiri and douroucoulis monkeys and the marmoset *Oedipomidas oedipus*. Attempts to infect the widow monkey (*Callicebus*) gave irregular results, and the local capuchin (*Cebus fatuellus*) in 4 instances failed to circulate enough virus to infect haemagogus mosquitoes. It thus seems likely that all monkeys are not equally important in the maintenance of virus cycles in nature.

Since the various species of mammals and mosquitoes and the various strains of virus show differences in behavior from place to place, it is considered important that transmission experiments be made with local materials in order to evaluate possible local transmissions mechanisms.

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# STUDIES ON CYCLIC PASSAGE OF YELLOW FEVER VIRUS IN SOUTH AMERICAN MAMMALS AND MOSQUITOES\*

## II. MARMOSETS (*CALLITHRIX PENICILLATA* AND *LEONTOCEBUS CHRYSOMELAS*) IN COMBINATION WITH *Aedes Aegypti*

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In the first paper of this series (1) the authors reviewed the literature on cyclic passage of yellow fever virus in neotropical mammals and arthropods, and reported the maintenance of a jungle strain of yellow fever virus by alternate passage through mosquitoes (*Aedes aegypti*) and a species of marmoset (*Callithrix aurita*), as well as Cebus monkeys (*Cebus versutus*). Subsequent to the submission of that paper for publication, Bates and Roca-García (2, 3) showed that jungle yellow fever virus of Colombian origin may be passed at will through *Saimiri sciureus caquetensis*, a Colombian species of "squirrel monkey," and *Aotus tririgatus*, a night monkey, with *Haemagogus capricornii*<sup>1</sup> interposed as vector.

During a recent study on the epidemiology of endemic jungle yellow fever in Brazil, the virus was isolated on four separate occasions from captured marmosets (*Callithrix penicillata*) (6). Though antigenetically analogous to other jungle strains in our possession, this strain differed in the following respects.

It derived from an area where the disease is endemic; it was isolated from wild, captured marmosets; and it had not undergone laboratory passage through any other species of animal.

In attempting to assess the rôle in transmission of the virus played by the two most common primates (*C. penicillata* and *Leontocebus chrysomelas*) found in the region where the virus was isolated, it became of interest to know whether this virus strain could be maintained by alternate serial passages through these animals and a suitable insect vector. With this object in view, the following experiments were carried out.

### MATERIALS AND METHODS

**Virus strain.**—The virus strain employed in these experiments was isolated from a sick marmoset trapped in August 1944 in a cacao grove near the edge of a small second-growth forest on the Fazenda Almada situated some 25 kilometers

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<sup>1</sup> In view of the more recent taxonomic description of *Haemagogus capricornii* by Cerqueira and Lane (4) and the studies on Colombian species of *Haemagogus* by Kumm, Osorno-Mesa, and Boshell-Manrique (5) it is probable that the species employed by Bates and Roca-García was a variety, or subspecies, of *spgazzinii*, which has been designated by Kumm *et al* as *Haemagogus spgazzinii falco*.

from the town of Ilheus in the southeastern section of the State of Bahia, Brazil (6). This virus (designated as the "Almada" strain) was serologically identified as yellow fever virus. The incidence of infections among the rural inhabitants over a series of years, indicates that jungle yellow fever is endemic in this region.

The virus is highly infectious to mice when inoculated intracerebrally, but many of the infected mice survive and are subsequently resistant to a challenge dose of French neurotropic virus. While survival of infected mice has been observed with other jungle strains, it occurs somewhat more frequently with the Almada strain.

The cyclic passages were initiated by infecting *Aedes aegypti* on a marmoset (*C. aurita*) which had been inoculated subcutaneously with a suspension of liver from the second passage *C. penicillata*.

*Vertebrate hosts.*—Two species of marmosets (*Callithrix penicillata* (E. Geoffrey) and *Leontocebus chrysomelas* (Kuhl)) were tested. The former, *C. penicillata*, is by far the most prevalent species of primates in the Ilheus region. It is found not only in the old dense forests but also in shaded cacao plantations and interspersed second-growth forests. The *Leontocebus* is found principally in the more extensive older types of forest and, while much less numerous than *C. penicillata*, was observed and captured more frequently than any of the remaining species of primates.

All of the animals utilized in the experiments were captured in the vicinity of Ilheus. Upon receipt at the field laboratory they were bled by heart puncture and the sera were examined for the presence of antibodies neutralizing yellow fever virus. Non-immune healthy animals were shipped by air-express to the central laboratory at Rio de Janeiro, where they were again bled and tested for immunity. Thus, the animals were submitted to two preliminary immunity tests, the first at the time of capture, and the second three or more weeks later. Only those shown to be non-immune on both occasions were used in the experiments.

*Insect vectors.*—Laboratory reared *Aedes aegypti* (Linnaeus) mosquitoes were used as the intermediate insect vector. They derived from a colony established in 1942 from female mosquitoes captured in the State of Rio de Janeiro.

*Virus determination.*—Quantitative determinations of circulating virus in the animals were made by titrating their blood sera in tenfold dilutions and inoculating each serum dilution intracerebrally into a group of six white Swiss mice. The presence of the virus in mosquitoes was confirmed either by allowing the mosquitoes to feed on two-day-old mice, or by triturating five or more mosquitoes in proportion of one mosquito to 0.1 cc. of diluent and inoculating the emulsion intracerebrally into adult mice. All dilutions were made in 0.85 per cent saline solution containing 10 per cent normal human serum.

*Tests for immunity.*—The intracerebral technique described by Theiler (7) was utilized. The mice used in the titrations were between twenty-one and twenty-eight days of age and the unit of virus (French neurotropic) approximated 100 MLD.

TABLE 1

Callithrix penicillata-Aedes aegypti Cycle Almada Strain of Yellow Fever Virus

CYCLES	CALLITHRIX PENICILLATA						AEDES AEGYPTI	
	Manner of infection	Circulating virus		Neutralization test		Day of death or survival	Lot	Test for virus
		Day	Titer	Pre	Post			
1	M1 By bite of 4 <i>A. aegypti</i> 23 days after feeding on a third laboratory passage marmoset ( <i>C. aurila</i> )	2	0	Negative	Positive	Survived		
		3	$10^{2.0}$					
		4	$10^{4.8}$				a	Positive by bite
		5	$10^{2.2}$				b	Not tested
		6	0				c	Negative by bite
		7	0					
	M2 By bite of 10 <i>A. aegypti</i> 27 days after feeding on a third passage marmoset ( <i>C. aurila</i> )	3	$10^{2.5}$	Negative		8	a	Positive by bite
		5	$>10^{2.0}$				b	Positive by bite
		6	$>10^{2.0}$				c	Positive by bite
		7	$>10^{1.0}$					
	M3 By bite of 25 <i>A. aegypti</i> Lot a, 24 days after feeding on M1.	2	$>10^{5.0}$	Negative	Positive	Survived		
		3	$10^{5.2}$				a	Positive by bite
		4	$10^{3.4}$				b	Positive by bite
		5	$10^{2.3}$				c	Negative by bite and inoculation
		6	$10^{1.6}$				d	Negative by bite and inoculation
		7	$<10^{1.0}$					
	M4 By bite of 81 <i>A. aegypti</i> Lot a and b, 25 days after feeding on M2	2	$10^{4.4}$	Negative		6		Not tested
		3	$10^{6.5}$					
		4	$10^{4.8}$					
		5	$10^{2.3}$					
2	M5 By bite of 19 <i>A. aegypti</i> Lot a, 25 days after feeding on M3	2	$10^{2.4}$	Negative		8		
		3	$10^{4.3}$				a	Positive by bite
		4	$10^{3.4}$				b	Positive by bite
		5	$10^{2.7}$				c	Not tested
		6	$<10^{1.0}$				d	Not tested
		7	$10^{2.3}$				e	Not tested
3	M6 By bite of 25 <i>A. aegypti</i> Lot b, 24 days after feeding on M5	2	$10^{4.3}$	Negative		5	a	Not tested
		3	$10^{7.0}$				b	Positive by bite
		4	$10^{7.0}$				c	Positive by bite
4	M7 By bite of 21 <i>A. aegypti</i> Lot b, 18 days after feeding on M6	3	$>10^{1.0}$	Negative		5	a	Positive by bite
		4	$10^{5.0}$				b	Positive by bite

TABLE 1—*Continued*

CYCLES	CALLITHRIX PENTICILLATA					AEDES AEGYPTI		
	Manner of infection	Circulating virus		Neutralization test		Day of death or survival	Lot	Test for virus
		Day	Titer	Pre	Post			
5	M8 By bite of 24 <i>A. aegypti</i> Lots a and b, 17 and 18 days after feeding on M7	2	10 <sup>5.0</sup>	Negative		7	a	Positive by bite
		3	10 <sup>7.0</sup>				b	Positive by bite
		4	10 <sup>6.8</sup>				c	Positive by bite
		5	10 <sup>8.0</sup>				d	Positive by bite

Note: No infections occurred among the mice inoculated with the 10<sup>-1</sup> and 10<sup>-2</sup> dilutions. One mouse died in the group which received the 10<sup>-3</sup> dilution. The same material titrated in two-day-old mice gave a titer of 10<sup>2.3</sup>.

*Technique employed in host-vector cycles.*—The technique employed in carrying through the host-vector cycle has been described previously (1). The cycles were initiated by exposing the first animal of the series to the bite of infected mosquitoes. On successive days following the trial infection, usually ranging from the second to the seventh day, lots of normal mosquitoes were permitted to feed on the animal. At the same time blood was withdrawn from the animal, and the serum was titrated for virus content. After an incubation period of approximately three weeks, mosquitoes which had fed during the period when the virus was circulating were allowed to bite the next normal animal in the series, and that animal was in turn exposed to batches of normal mosquitoes and tested for circulating virus. Between feedings the mosquitoes were kept at temperatures between 20–34°C. An average of about twenty mosquitoes were used for each transmission. The animals that survived infection were tested for circulating antibodies three weeks after being exposed. Each lot of mosquitoes used for transmission was examined for virus as described above. The animals that died were autopsied, and their livers were examined for lesions characteristic of yellow fever virus infection.

Thus, the maintenance of the virus in the cyclic transmissions was determined by the presence of circulating virus in the animal host, by the presence of virus in the mosquitoes following a period of incubation, by histopathological changes in the livers of the animals that succumbed, and by the development of immunity in the animals that survived. It may be added, however, that marmosets do not consistently develop liver lesions characteristic of yellow fever infection, so this method of identifying infection is not dependable.

#### EXPERIMENTAL RESULTS

*Callithrix penicillata cycle.*—No difficulty was encountered in the alternate passage of the virus through *C. penicillata* and *Aedes aegypti*. Indeed, it appears



to be easier to maintain the virus by alternate passage through mosquitoes<sup>2</sup> and this marmoset than by direct syringe passage from marmoset to marmoset. The results of five cyclic passages are recorded in table 1 and fig. 1.

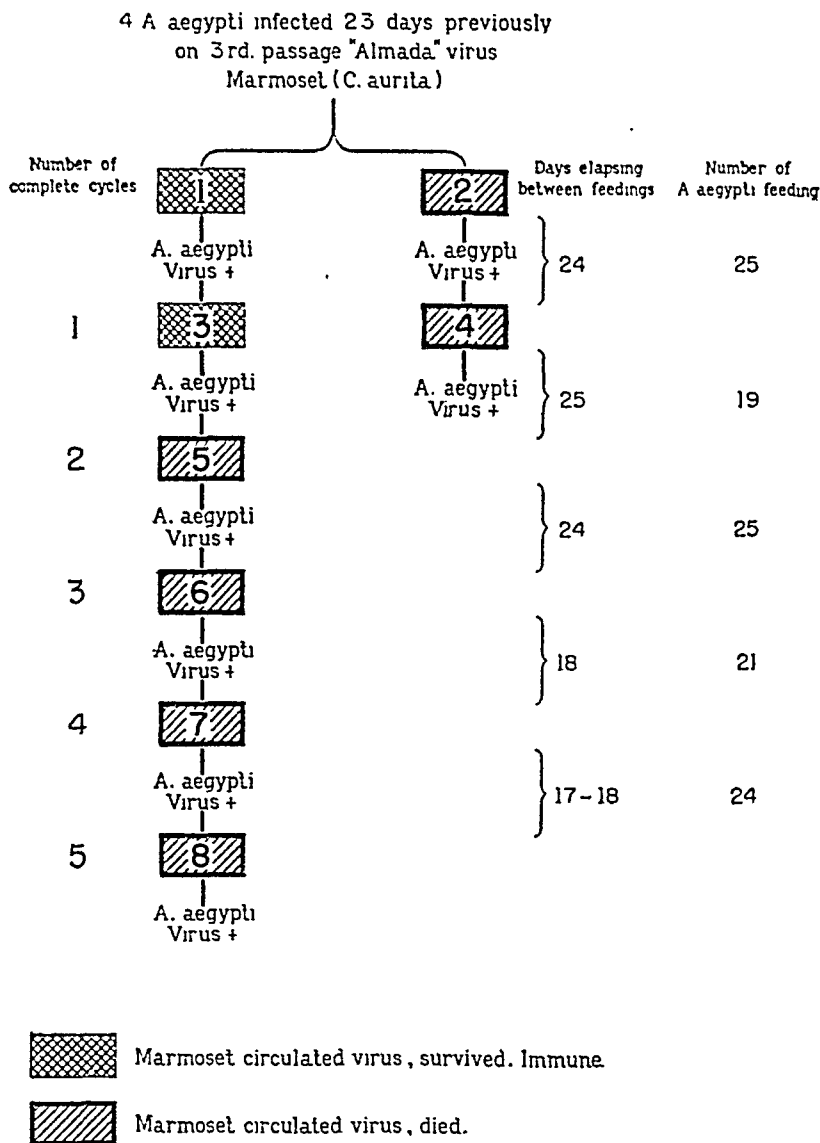


FIG. 1. RECORD OF CYCLIC PASSAGES OF YELLOW FEVER VIRUS (ALMADA STRAIN) THROUGH *CALLITHRIX PENICILLATA* AND *AEDES AEGYPTI*

All of the eight animals exposed to infected mosquitoes circulated virus, and six of the eight died. The two survivors developed immune antibodies. The

<sup>2</sup> We are indebted to Dr. T. P. Hughes of this laboratory for information on direct passage of the virus in *C. penicillata*. He encountered some difficulty in keeping the strain going by syringe passage in this animal. After several serial passages the virus appeared to become less virulent and was more difficult to transfer.

fact that only one of the six which died manifested characteristic liver lesions does not exclude virus infection as the cause of death. The time of death, in relation to exposure, the presence of the virus in the blood stream, and the failure of autopsy to reveal any other cause of death strongly suggest that all of these animals succumbed to infection with the virus.

17 *A. aegypti* infected 46 days previously  
on 3rd. passage "Almada" virus  
Marmoset (*C aurita*)

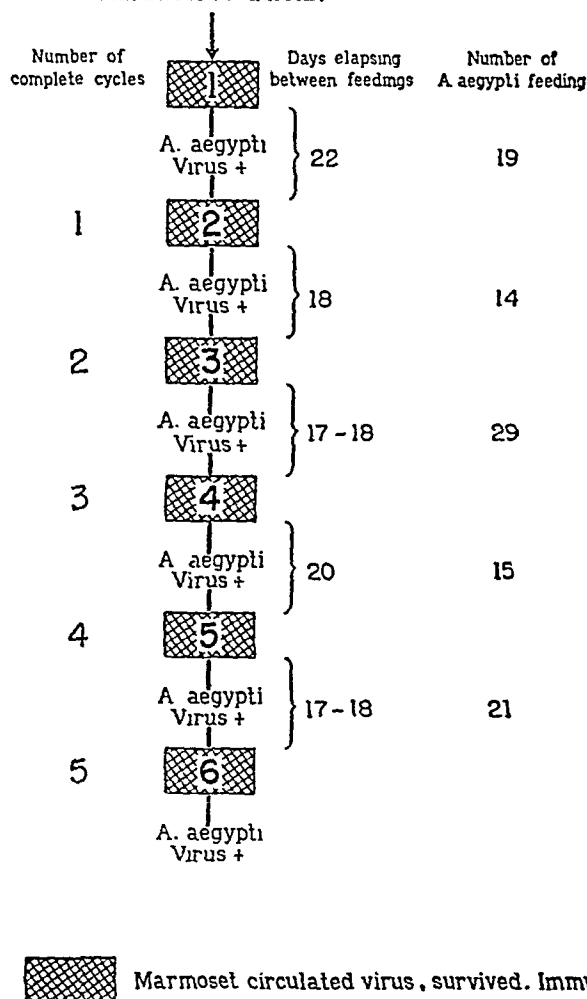


FIG. 2. RECORD OF CYCLIC PASSAGES OF YELLOW FEVER VIRUS (ALMADA STRAIN) THROUGH *LEONTOCEBUS CHRYSOMELAS* AND *AEDES AEGYPTI*

Virus was usually present in the blood stream on the second day following the feeding by infected mosquitoes and persisted through the fifth to the seventh day, or until death. The maximum concentration of circulating virus, as determined by titration in mice, varied from  $10^{4.3}$  to  $10^8$ . It should be noted, however, that, because of the variable lethality of this virus to mice, the quantitative estimates are relatively inexact and tend to fall below the actual virus content.

Nineteen separate lots of mosquitoes that had fed on infected animals were

TABLE 2

*Leontocebus chrysomelas*-*Aedes aegypti* Cycle Almada Strain of Yellow Fever Virus

CYCLES	LEONTOCEBUS CHRYSOMELAS						AEDES AEGYPTI	
	Manner of infection	Circulating virus		Neutralization test		Day of death or survival	Lot	Test for virus
		Day	Titer	Pre	Post			
	M1 By bite of 17 <i>A. aegypti</i> 46 days after feeding on a 3rd passage marmoset ( <i>C. aurita</i> )	1	10 <sup>1.1</sup>	Negative	Positive	Survived		
		2	10 <sup>4.5</sup>					
		3	10 <sup>6.0</sup>				a	Positive by bite
		4	10 <sup>5.2</sup>				b	Not tested
		5	10 <sup>5.7</sup>				c	Not tested
		6	10 <sup>2.2</sup>				d	Not tested
		7	10 <sup>1.2</sup>				e	Negative by bite and inoculation
		8	10 <sup>1.0</sup>					
1	M2 By bite of 19 <i>A. aegypti</i> Lot a, 22 days after feeding on M1.	2	10 <sup>4.3</sup>	Negative	Positive	Survived		
		3	10 <sup>6.2</sup>				a	Positive by bite
		4	10 <sup>4.2</sup>				b	Positive by bite
		5	10 <sup>3.5</sup>				c	Not tested
		6	10 <sup>3.7</sup>				d	Not tested
		7	10 <sup>2.5</sup>				e	Negative by bite and inoculation
2	M3 By bite of 14 <i>A. aegypti</i> Lot a, 18 days after feeding on M2	2	10 <sup>2.5</sup>	Negative	Positive	Survived		
		3	10 <sup>6.0</sup>				a	Positive by bite
		4	10 <sup>6.3</sup>				b	Positive by bite
		5	10 <sup>2.0</sup>				c	Not tested
		6	10 <sup>2.4</sup>					
		7	10 <sup>1.4</sup>					
3	M4 By bite of 29 <i>A. aegypti</i> Lots a and b, 17 and 18 days after feeding on M3	2	10 <sup>6.5</sup>	Negative	Positive	Survived		
		3	10 <sup>5.4</sup>				a	Positive by bite
		4	10 <sup>2.4</sup>				b	Positive by bite
		5	10 <sup>2.2</sup>				c	Not tested
		6	10 <sup>1.0</sup>				d	Not tested
		7	10 <sup>1.5</sup>				e	Negative by bite
4	M5 By bite of 15 <i>A. aegypti</i> Lot a, 20 days after feeding on M4	3	10 <sup>4.0</sup>	Negative	Positive	Survived	a	Positive by bite
		4	10 <sup>4.0</sup>				b	Positive by bite
		5	10 <sup>1.0</sup>				c	Negative by bite
5	M6 By bite of 21 <i>A. aegypti</i> Lots a and b, 17 and 18 days after feeding on M5	2	10 <sup>1.0</sup>	Negative	Positive	Survived		
		3	10 <sup>7.0</sup>				a	Positive by bite
		4	10 <sup>6.0</sup>				b	Positive by bite
		5	10 <sup>4.0</sup>				c	Positive by bite

tested for virus, and in all, save two, virus was demonstrated. These two lots of mosquitoes fed at a time when the titers of the circulating virus in the animals were low, 10<sup>2.3</sup> and 1<sup>6</sup>.

*Leontocebus chrysomelas* cycle.—Five host-vector cycles were completed (table

2, fig. 2) using *L. chrysomelas* as the animal host. The cycles were initiated by permitting mosquitoes infected from a *C. aurita* of the third laboratory passage to feed upon the primary *Leontocebus*. As there was no apparent diminution in the circulating virus after the fifth passage and the mosquitoes continued to be infected, the cycles were voluntarily discontinued.

The infection seems to run a milder course in this species of marmoset than in *C. penicillata*, since none of the six animals used in the experiment succumbed. However, all of the animals circulated virus following the infected mosquito feeding and all subsequently developed neutralizing antibodies against yellow fever. Circulating virus was present in all animals tested on the second day following exposure and continued to circulate through the fifth to the eighth day; with the exception of one animal the titer reached or exceeded  $10^6$  at some time during the course of the infection.

Mosquito lots fed during the early stages of the infection consistently contained virus. It is of interest to note that mosquitoes feeding on the seventh day, and in one instance on the fifth day, following exposure of the animal failed to show virus after the period of incubation, although the virus was still circulating in diminished concentration at the time of feeding.

#### DISCUSSION

The epidemiological studies which stimulated these experiments indicated that *C. penicillata*, and possibly other primates including *L. chrysomelas*, were the principal if not the sole vertebrate hosts of the virus in the region where the studies were conducted (8). This deduction was based upon (a) the isolation of the virus on four separate occasions from captured *C. penicillata*, (b) the association of specific immunity to yellow fever in the human population with that in captured primates, and (c) the absence of any convincing evidence incriminating other orders of vertebrates. The demonstration that the strain of jungle yellow fever which was isolated may be propagated at will by alternate passage through either *C. penicillata* or *L. chrysomelas* and a suitable vector, lends further support to this view.

The field studies, above referred to, incriminated *Haemagogus spegazzinii* as the probable vector. The virus was isolated from captured specimens of this species during the period in which it was obtained from marmosets, and there was a definite correlation between the prevalence of this mosquito and the incidence of immunity to yellow fever among both humans and marmosets. It would have been more in keeping with what probably takes place in the forest to have used *H. spegazzinii* instead of *A. aegypti* in these transmission experiments. The latter were employed as a matter of convenience since it has not been possible to rear *H. spegazzinii* in the laboratory and thus be assured of an adequate and constant supply. *H. spegazzinii*, as well as other species of *Haemagogus*, have been shown to be effective vectors of the virus, and it may be presumed that *H. spegazzinii* would have served equally as well as *A. aegypti*. Moreover, the purpose of these experiments was to ascertain whether the two species of marmosets

were suitable vertebrate hosts for cyclic transmission of the virus, irrespective of the vector.

#### SUMMARY

A strain (Almada) of endemic jungle yellow fever virus was easily maintained in a continuous host-vector cycle by employing *C. penicillata* and *L. chrysomelas* as vertebrate hosts in combination with *A. aegypti* as insect vector.

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# • THE DISPERSAL OF *Aedes albopictus* IN THE TERRITORY OF HAWAII

DAVID D. BONNET<sup>1</sup> AND DOUGLAS J. WORCESTER<sup>2</sup>

Since the discovery of the importance of mosquitoes as vectors of disease, numerous studies have been made on the distances the various species travel from a breeding source or natural resting place. This distance has been variously named, "*flight*" (Zetek, 1913; Shannon and Davis, 1930), "*distance of flight*" (Le Prince and Griffiths, 1917), "*flight range*" (Russell and Santiago, 1934), "*flying radius*" (Avé Lallemant, Soerono and Soekaria, 1931) and "*range of dispersion*" (Eyles and Bishop 1943).

An excellent review of the methods used in determining flight range and a discussion of factors influencing the distance is given by Russell and Santiago, 1934. Most of the studies have been made on species of *Anopheles* mosquitoes, particularly the important vectors of malaria. (Cf. Zetek, 1913; Le Prince and Griffiths, 1917; Kumm, 1929; Avé Lallemant, Soerono and Soekaria, 1931; Wallace, 1939; Adams, 1940; Eyles and Bishop, 1943.) Other species, particularly the pest forms, have also been studied. (MacCreary and Stearns, 1937; Stage, Gjullin and Yates, 1937; Curry, 1938; Clarke, 1937, 1943.) *Aedes aegypti*, one vector for yellow fever and dengue fever, has been stated to travel only the relatively short distance of 75 to 100 yards. Ordinarily this appears to be true and this range is widely accepted by those dealing with *Aedes aegypti* from epidemiological and control aspects. Soper (1937) states that the presence of adults with a careful analysis of the relative density of the two sexes, makes "it possible, in most cases, to localize hidden breeding within a radius of 25 or 30 yards." However, Shannon and Davis (1930) have shown that *Aedes aegypti* is capable of a sustained flight of one kilometer (0.62 miles) over water. From a release on land of over 20,000 stained specimens, two were recovered at distances of 325 meters (355 yards) and 330 meters (361 yards) respectively. Ninety-five other stained specimens were captured at intermediate distances, the majority at distances less than 120 meters (131 yards).

*Aedes pseudoscutellaris* (Theobald) has been stated to have a limited flight range (Buxton and Hopkins, 1927). More recently during a study of the natural filarial infection rate in Samoa, Byrd *et al.*, (1945) have shown that the incidence of infection in the mosquitoes in a village was as high as 45 per cent. This rate of infection decreased markedly at the periphery of the village. At 50 yards from the edge of the village the incidence was 4 to 5 per cent. At greater distances infected mosquitoes are rarely found. This has been cited as evidence of a flight range of 100 yards or less.

*Aedes albopictus* (Skuse) has been demonstrated to be a vector of dengue (Simmons, St. John and Reynolds, 1930; Snijders, Dinger and Schuffner, 1931). It has also been shown experimentally to be a possible vector for yellow fever.

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(Dinger *et al.*, 1929; Snijders, 1931.) Although Craig and Faust, (1930) on the basis of experimental evidence of other workers, list *Aedes albopictus* as an "efficient transmitter" and a "good incubator" of yellow fever virus, a more recent listing by the same authors (Craig and Faust, 1945), states that it is an "inefficient transmitter." The biology of this species is reported to be very similar to *Aedes aegypti*, but relatively little study has been made of its habits and life history. (Cf. Senior-White, 1934; Sen, 1924, 1935; Toumanoff, 1939.) Usinger (1944) has reviewed and compared the biology of *Aedes albopictus* with *Aedes aegypti*. The flight range of *Aedes albopictus* is reported by Senior-White (1934) to be quite limited. In spite of the medical importance of this species, no experimental evidence has been obtained to demonstrate the normal range of dispersal.

The present paper is a report of twelve release-recapture experiments with *Aedes albopictus* in Oahu, Territory of Hawaii.

#### METHODS

The methods used in these experiments are those commonly used in similar experiments. *Aedes albopictus* larvae were obtained from eggs laid in jars in a breeding cage in the laboratory. The cage was stocked with large numbers of males and females, and the adults were fed on raisins and sugar water with a blood meal every other day. In the breeding cage which measured only 18" x 18" x 20", copulation occurred readily and a steady supply of fertile eggs was available at all times. The eggs were removed when needed and high percentages were induced to hatch by the addition of a few grains of rice to the water. (Cf. Gjullin, Hegerty and Bollen, 1941.) The larvae were raised in shallow pans to which fresh water was added periodically. They were fed on rice grains, dog biscuit, yeast, bread crumbs or hay infusions. Of these foods, rice grains with yeast produced the most robust specimens, but not infrequently a heavy scum formed on the surface of the water, interfering with the growth of the larvae. Powdered stale bread crumbs, which also produced healthy, good-sized specimens, did not form this scum. Each day the pupae were removed from the pans, counted and placed in a cage which contained a growing plant, raisins and sugar water as food for emerged adults. When the number of mosquitoes that had emerged were sufficiently numerous, the cage was removed from the laboratory, the food taken out and the mosquitoes dusted with a mixture of flour and dye in order to mark the specimens. The dyes used were Auramine "O," Pyrazol Red, Gentian Violet, Malachite Green, Methyl Orange, Toluidine Blue, Pyrazol Violet and Chloramine Red. Of this group, Chloramine Red was the only dye which proved unsuccessful. The colors were mixed with flour in the proportion of 1 to 50, and the dusting accomplished with a hand duster (Root, Jr.). After dusting, the cage was transported to the experimental area and the mosquitoes released. Tests were made on the efficiency of this method of marking, and between 90 and 100 per cent of the mosquitoes in the cage demonstrated the stain when dipped into dye solvent.<sup>3</sup>

<sup>3</sup> The dye solvent formula: 70% alcohol—3 parts, glycerin—1 part, chloroform—1 part.



The release was usually made at the beginning of the week and collections were made during the remainder of the week. A different color was used on successive weeks in most instances, so that the date of release could be accurately determined. In cases where duplication of colors was necessary, the same point of release was used and the more recent date used in calculating time. Hand catching with the aspirator designed by Dr. Robert Matheson of Cornell University, was utilized, as various types of traps had been tried without success. The capturing was done by the authors, with occasional assistance of others.

In the use of the aspirator, various techniques were tried, the most successful being the production of a slow steady suction. With this method it was found that many mosquitoes could be caught on the wing and it was not necessary to wait for these wary biters to settle on the exposed skin. The mosquitoes hovered in clusters, particularly around the legs, beneath the knees or at the side as one made the collections. The majority of the individuals in these swarms were males and they rarely alighted. It was possible when sufficient numbers were present to catch both male and female mosquitoes at the rate of 200 per hour per man. This method of catching accounts for the abnormal ratio of males to females. (Cf. table 3.)

The stations were visited at least once a week and more often when possible. The captured specimens were killed with carbon tetrachloride vapor and placed in labeled test tubes. Later each specimen was individually checked as to species and sex and examined under a dissecting microscope as they were dipped into dye solvent. Marked specimens were immediately apparent due to the "explosion" of color as the small adhering dye particles dissolved in the solvent. In no case was there any doubt as to the color involved. The presence of particles of different colors that did not show this "explosive reaction" were not considered, since pollen grains and other particles were frequently observed. The dye particles will stick on all parts of the body of the mosquito but are found more frequently on the head, antennae, cervical sclerites, coxae and near the pedicel of the wings.

#### THE EXPERIMENTAL AREA

This series of experiments was performed during the months of August, September, October and November 1944 at the Territorial Board of Agriculture and Forestry Nursery in Makiki Valley, Oahu, T. H. This small, narrow, y-shaped valley lies within three miles of the center of the city in the Koolau mountain range. The valley floor has an approximate elevation of 300 feet and is in a region of moderate rainfall. The average temperature and humidity for Honolulu during the months of the experiment were 77° F. and 65 per cent relative humidity. The prevailing direction of the wind is from the head of the valley in the mountains towards the lower end of the valley. The stream within the valley (Makiki Stream) is formed by the confluence of two smaller streams (Kaneaole and Maleka), the Maleka Stream being intermittent in character. The areas close to the streams are heavily vegetated while the remainder of the floor of the valley is semi-cultivated by the nursery and consists of open areas

where the principal vegetation is young seedlings. The moderately steep slopes and the lower end of the valley are covered with a thick stand of haole koa (*Leucaena glauca*). The head of the valley is forest reserve and consists of kukui (candle-nut) trees (*Aleurites* sp.), coconut palm trees (*Cocos* sp.), some banyans (*Ficus* sp.), kiawe (*Prosopis* sp.), Norfolk pine and other miscellaneous introduced trees.

Also present are a few scattered patches of native cultivation with bananas, coffee, papaya, upland taro (Ape), ti (*Cordyline terminalis*), etc. The undergrowth, where present, consists principally of palm grass (*Setaria palmifolia*). There are approximately 10 Portuguese-Hawaiian families living in unscreened and open houses in this area. In addition to the human source of blood, there were chickens, pigs, dogs, cats, rats, mongooses and wild birds present in the vicinity. For general details of this area, consult the map presented in figure 1

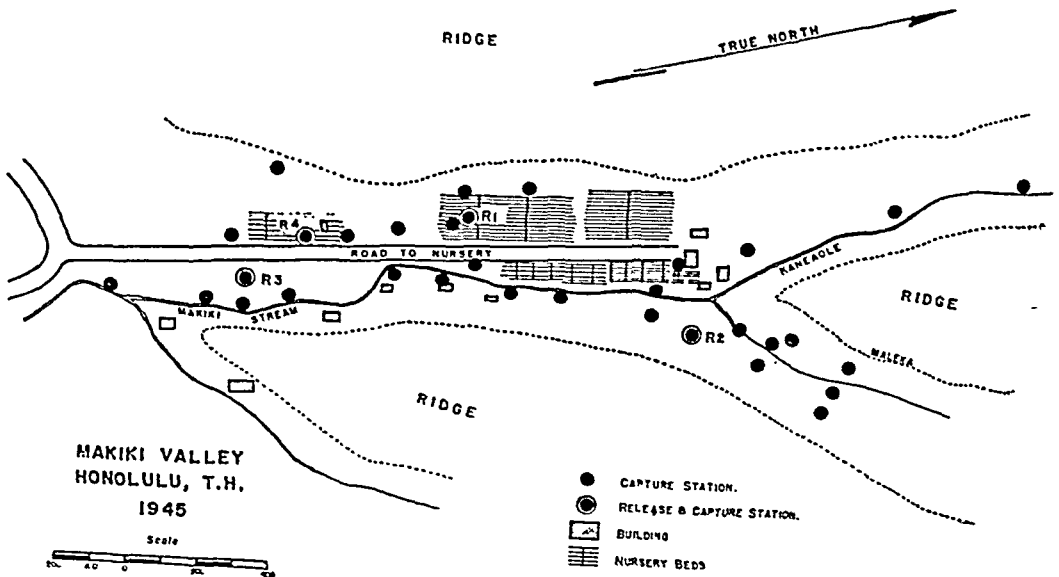


FIG. 1

Four different points of release were used which are labeled R1, R2, R3 and R4, respectively. Of these, only R4 gave unsatisfactory returns. R1 was located in the center of a rather wide open area with only low vegetation. The nearest group of trees was at a distance of 30 yards. R2 was in the center of a thick, well-shaded vegetation area. The air at R2 was cooler and more moist than at R1, and a large natural mosquito population was always present. R3 was located in the center of a large growth of ti plants near the mouth of the valley. There was a large natural mosquito population present but not in as great numbers as at R2. R4 was located near the road but was used only once as the location did not prove satisfactory. The capture stations were located wherever mosquitoes were found in sufficient quantities to permit capture, and at varying distances from the point of release. The locations of these stations are indicated on the map in figure 1.

## RESULTS AND DISCUSSION

Presented in table 1 is a summary of the individual releases, giving the number of stained specimens released, the number of specimens retaken or recaptured and the minimum number of days from time of release and the distance traveled for the recaptures. It will be noted that the numbers retaken for any release ranged between 0.09 per cent to 33.3 per cent. The percentage of all releases recaptured was 3.8 per cent. It is obvious that an explanation is required for the abnormally high return for the release of Toluidine Blue marked mosquitoes on September 11, 1944. It will be noted that 53 out of the 100 recaptures were taken after two days at the point of release. This is not an unexpected result since the point of release was a situation where mosquitoes were commonly present and where the ecological conditions appeared to be ideal for the mosquitoes. It is more difficult to offer an explanation for the 18 and 19 marked specimens caught at distances of 145 yards and 108 yards from the point of release, respectively. Although one might suspect an error in technique, it is the considered opinion of the authors that this return is correct, and that it may be accounted for by a wholesale movement of a swarm from the point of release to the recapture stations. At the time of recapture at these stations, there was a relatively small local unstained population; hence, the marked specimens became proportionately more available for capture without dilution. The low returns in the cases of the other releases may be due to collections being made at the recapture stations on the day or days that the swarm was not present, while the few marked captures represent stragglers from a swarm. This is further substantiated by the casual observation that there is considerable variation in the numbers of mosquitoes present at the collecting stations. Occasionally during the periods of collection, the collector was very actively capturing mosquitoes when suddenly no more mosquitoes were present. Later, another or the same group appeared and collecting was resumed. It was determined by experience that approximately 15 or 20 minutes of collecting at any station was the practical limit and that there was little use in remaining after that period of time. However, this was not due to the capture of all the available mosquitoes. The capturing did not gradually diminish, but the mosquitoes suddenly and completely disappeared. A return to the station later, on the same day, would usually produce an additional return as large as the earlier capture. Gross (personal communication, 1945) states that *Aedes aegypti* have been observed in Key West, Florida, moving as a swarm from the front of a dwelling to the rear where a cistern was located. Further experiments are necessary to substantiate the swarm movements in *Aedes albopictus*.

Wind, which is frequently incriminated as responsible for distribution, is of little account in the case of this species. *Aedes albopictus* flies close to the ground and was never observed flying in a strong breeze. They were observed dropping in flight and clinging to grass or bush when moderate or heavy gusts of wind were present. In general, the only effect of wind will be to orient the direction of flight, for this species was observed flying into the wind when the velocities were extremely low.

TABLE 1

DATE RELEASED AND COLOR	NUMBER RELEASED	% OF RELEASED RETAKEN	NUMBER RETAKEN	MINIMUM DAYS SINCE RELEASE	DISTANCE  yds.
8-3-44 Pyrazol Red	350	3.4	6 4 1 1	0 1 4 7	30 62 62 52
8-14-44 Pyrazol Red	700	2.3	8 1 2 3 1 1	1 1 2 2 4 9	52 177 52 177 91 177
8-21-44 Auramine "O"	550	2.0	1 1 2 5 2	0 0 1 1 7	52 177 62 30 177
8-30-44 Auramine "O"	500	0.6	1 1 1	19 20 21	219 58 232
9-4-44 Pyrazol Brilliant Violet	275	3.3	4 1 3 1	9 9 14 14	0 49 42 75
9-11-44 Toluidine Blue	300	33.3	53 3 2 3 18 19 1 1	2 2 7 7 8 8 9 14	0 49 132 75 145 108 82 145
9-18-44 Methyl Orange	800	0.5	2 1 1	3 9 10	181
9-25-44 Chloramine Red	1000	No recoveries			
10-2-44 Malachite Green	475	0.6	1 1 1	1 3 9	112 475 35
10-4-44 Auramine "O"	1100	0.09	1	7	35

TABLE 1—*Continued*

DATE RELEASED AND COLOR	NUMBER RELEASED	% OF RELEASED RETAKEN	NUMBER RETAKEN	MINIMUM DAYS SINCE RELEASE	DISTANCE  <i>yds.</i>
10-23-44 Genetian Violet	600	1.5	3	4	30
			2	4	52
			1	4	62
			2	7	132
			1	10	35
11-13-44 Toluidine Blue	450	3.3	3	1	30
			1	1	35
			4	1	52
			7	3	30

The relation between the number of days from the time of release and the date of capture to the distance traveled is given in table 2. The maximum elapsed time was 21 days involving a distance of 232 yards. The mean distance for each day was calculated by dividing the total distance that the mosquitoes captured that day had traveled, by the number of these mosquitoes. Similar calculations were made in determining the mean distance for five day groups. The mean day for each group was determined by multiplying the number of mosquitoes recaptured by the number of days since release and dividing the sum of the products by the total number of mosquitoes in the group. The mean day was then divided into the mean distance for each five day group in order to obtain the mean rate in yards per day per mosquito. The mean rate ranges from 7.0 yards per day to 20.2 yards per day. Treating the entire series as a single group, similar calculations have been made. Of the total of 183 mosquitoes recaptured on different days up to 21 days, the mean day was 4.5. The mean distance for all recaptures was 68.7 yards. The mean rate, determined by dividing the mean distance by the mean day, is 15.2 yards per day. It is tempting to draw far reaching conclusions but they must be utilized with extreme caution. The limitations are severe and the figures are meaningless unless a large number of returns can be accumulated, under conditions where extraneous influences such as wind, local preferences, breeding locations, meal locations, etc. will not warp the dispersal picture. Furthermore, such extensive averaging of figures and weighing of results serves more often to obscure inequalities than explain them. An examination of the mean distance for the five day groups gives a slight indication of the rapidity with which this dispersal is occurring. The mean distances traveled in 5 days, 10 days and 15 days were: 36.3 yards, 113.8 yards and 103 yards, respectively. The rate of dispersal is apparently decreasing during the same periods. Little weight should be given to the other groups, since the returns were too low to be of significance. It is necessary to remember that the points of recapture do not necessarily represent the flight of the mosquito. The mosquito will

rarely, if ever, fly in a "bee-line." The distance and direction between the point of release and the point of capture represents the final components of distance and directions and does not take into account the side excursions, back-tracking or days when no flying occurred.

Some authors have utilized the term "flight" to represent the distance traveled from the point of release to the point of recapture. The use of this term is only justified if the mosquito has traveled a long distance in a sustained flight or series

TABLE 2

DAYS AFTER RELEASES	NUMBER RECAPTURED	MEAN DISTANCE PER DAY	MEAN DAY PER 5 DAY GROUP	MEAN DISTANCE PER MOSQUITO FOR 5 DAY GROUPS	MEAN RATE PER MOSQUITO PER DAY FOR 5 DAY GROUPS
		<i>yards</i>		<i>yards</i>	<i>yards</i>
$\frac{1}{8}$	8	49.6			
1	29	53			
2	61	13.6	1.8	36.3	20.2
3	10	105			
4	8	50			
5	0				
6	0				
7	11	109			
8	37	120	7.9	113.8	14.4
9	9	94			
10	2	188			
11	0				
12	0				
13	0		12.9	103.0	8.0
14	5	69			
15	0				
16	0				
17	0				
18	0				
19	1	218	19.0	218	11.5
20	1	57			
21	1	232	20.5	144.5	7.0
Entire Series.....	183	68.7	4.5		15.2

of flights in a definite direction. The ordinary wandering excursions and movements from feeding locations to breeding spots and natural resting places is more correctly termed dispersal, and it is ordinarily this movement which is determined by recapture-release experiments.

The total capture at the different distances from the points of release and the number of marked specimens recaptured at each distance is given in table 3. Since, on the basis of an estimated life span of one month, there is an expectation of catching marked mosquitoes up to 30 days after the release, the total captures

TABLE 3

DISTANCE IN YARDS	NO. CAPTURED		TOTAL CAPT.	NO. MARKED CAPTURED		TOTAL CAPT.	RATIO TOTAL CAPT./TOTAL MARKED
	Male	Female		Male	Female		
0	1,280	454	1,734	45	12	57	30
30	226	166	392	7	17	24	16
35	219	99	318	3	1	4	80
42	118	66	184	3		3	61
49	599	133	732	2	2	4	183
52	957	273	1,230	14	4	18	68
58	191	122	313	1		1	313
62	421	217	638	2	6	8	80
75	254	157	411	3		3	137
82	620	209	829	1		1	829
91	690	206	896		1	1	896
108	211	137	348	16	3	19	18
112	282	156	438	1		1	438
128	110	49	159				
132	1,110	366	1,476	3	1	4	369
138	36	9	45				
145	500	119	619	15	4	19	33
167	14	9	23				
173	34	38	72				
177	3,340	1,152	4,492	6	2	8	562
181	383	159	542	2		2	271
200	371	114	485				
207	294	202	496				
213	39	23	62				
219	580	272	852	1		1	852
228	36	22	58				
232	114	82	196		1	1	196
250	1,932	732	2,664	1		1	2,664
273	326	78	404				
283	182	106	288				
292	109	23	132				
305	678	363	1,041				
317	118	40	158				
334	512	147	659				
339	274	114	388				
348	829	389	1,218	1		1	1,218
380	61	34	95				
402	168	57	225				
421	150	82	232				
437	90	31	121				
450	417	147	564		1	1	564
458	154	49	203				
469	241	139	380				
475	763	435	1,198	1		1	1,198
498	116	78	194				
533	201	155	356				
581	384	307	691				
644	118	40	158				
692	118	40	158				
Total .....	20,970	8,597	29,567	128	55	183	162

have been summed for 30 days after each release at each distance. Since releases were made approximately once a week, some catches refer to more than one release, and hence are duplicated in obtaining the totals. This gives an abnormally large number when figured as a grand total but should not lessen the accuracy of the calculation. Since each release was marked with a different color, the stained recaptures are referred to only one release and there is no duplication in the total of marked specimens.

It will be noticed by casual examination of table 3 that the great majority of returns were at distances of less than 100 yards and that very few returns occurred at distances greater than 200 yards. The maximum distance recorded is a distance of 475 yards. It is tempting to divide the total number captured by the number of stained mosquitoes in order to determine the number of mosquitoes it is necessary to catch before one can expect to capture a marked specimen. This has been done and the results are placed in the last column of table 3. In a general way it will be observed that the further the capturing station is from the point of release the greater will be the number of mosquitoes which must be captured in order to get a marked one. This would be expected merely on the basis of dispersal in relation to distance. However, there are a number of factors which prevent one from considering these figures too seriously. The total number of mosquitoes captured at each station will depend upon the total natural population at that station. If this natural population is high, the ratio of total captured mosquitoes to marked mosquitoes will also be high, irrespective of the distance from the release point. Similarly, an area free of mosquitoes may show an abnormally low rate if close enough to the point of release and if the capture is done during the period of initial dispersion after a release. In some instances it was observed that the mosquito population as measured by the number captured in 15 minutes by one man varied considerably from day to day. This would mean that the day of capture and particularly the time of day would influence this ratio. Other factors which would influence the rate of dispersion would include the ecological nature of the release point, the distance to a blood meal, the distance to a water source for egg deposition and the distance to a natural resting place. It is obvious that these mosquitoes can be forced to fly greater distances than they normally would if released at a point distant from any or all of these enumerated items. Such a condition would be found in the release from a vessel off-shore or a release in the center of an area unsuited for mosquitoes. Such experiments, although of value in determining a maximum distance, do not give any information on the ordinary rate of dispersion, which is the value desired for epidemiological and control purposes. Although no evidence is presented which shows that they would not go beyond the maximum distance here recorded, it would appear that only a few could travel greater distances unless transported artificially. It can be deduced from the figures presented here that the ordinary range of activity is 200 yards or less.

#### CONCLUSION

It is concluded on the basis of the above material that the distance which *Aedes albopictus* will normally travel during its lifetime will be 200 yards or less.



This distance will be determined by a large number of factors which make a statistical analysis of the data of questionable value. Wind is probably of less importance in the case of *Aedes albopictus* than is ordinarily believed. It is suggested that *Aedes albopictus* may travel in swarms but further experiments are necessary to establish this suggestion as factual. The average rate of dispersal has been determined at approximately 15 yards per day. This figure is an average of all components of distance and direction and is not a measure of straight flight range.

Release-recapture experiments should be considered as dispersal experiments unless the distance traveled is of sufficient magnitude to justify the use of the word flight.

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# STUDIES ON IMPORTED MALARIAS

## 5. TRANSMISSION OF FOREIGN PLASMODIUM VIVAX BY ANOPHELES QUADRIMACULATUS<sup>1</sup>

MARTIN D. YOUNG,<sup>2</sup> JOHN M. ELLIS<sup>3</sup> AND TRAWICK H. STUBBS<sup>4</sup>

### INTRODUCTION

It has been previously shown (Young, et al., 1946) that the mosquitoes of the southern United States were susceptible to foreign malarial relapsing in returned troops and that these malarial relapses developed to the infective stage in the insects. A preliminary report (Young, et al., 1945) indicated that the infected mosquitoes could successfully transmit the foreign malarial relapses.

This report will present the detailed observations on the ability of *Anopheles quadrimaculatus* to transmit various foreign malarial relapses to white and Negro patients. The patients were neurosyphilitic, with the exception of 14 white men who were volunteers on a drug testing program. The infections in these mosquitoes originated from the relapsing cases reported by Young, et al. (1946).

### METHODS

Mosquitoes from a lot proven to have sporozoites in the salivary glands were applied to the patients selected. The mosquitoes were dissected after feeding and the number with sporozoites determined.

Starting several days after the biting day, blood smears were made daily and examined for parasites. Temperature readings were made at regular intervals, usually every four hours during the afebrile state and hourly during fever periods.

An effort was made to determine whether the patient had had malaria previously, either natural or induced.

### OBSERVATIONS

*Total Transmission Attempts.* Transmission was attempted on 186 patients as shown in table 1.

<sup>1</sup> Contribution from the Imported Malaria Studies program of the Office of Malaria Investigations, National Institute of Health, and the Office of Malaria Control in War Areas.

The following hospitals cooperated by making neurosyphilitic patients available: Harmon General, Moore General, South Carolina State, Milledgeville (Ga.) State, North Carolina State at Morganton and Raleigh, and the University Hospital at Augusta, Ga. To these, and especially to Harmon General, Moore General, and the South Carolina State Hospitals, which also furnished laboratory quarters, we express appreciation. We are indebted also to the Office of the Surgeon General, U. S. Army, whose active interest made the program possible.

<sup>2</sup> Sanitarian (R) U. S. Public Health Service

<sup>3</sup> P. A. Sanitarian (R) U. S. Public Health Service

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TABLE 2

*Transmission of foreign vivax malarias by A. quadrimaculatus to white and negro patients. Arranged by origin of malaria. (First inoculations only. No reinoculations included)*

ORIGIN OF STRAIN	PATIENTS					
	White			Negro		
	Tried	Infected	Per cent infected	Tried	Infected	Per cent infected
Guadalcanal.....	37	31	83.8	9	3	33.3
New Guinea.....	80	76	95.0	9	1	11.1
Total Pacific.....	117	107	91.5	18	4	22.2
North Africa.....	7	6	85.7	4	1	25.0
Sicily & Italy.....	26	25	96.2	13	5	38.5
Total Mediterranean.....	33	31	93.9	17	6	35.3
Burma.....	1	1	100.0	0	0	0.0
Total.....	151	139	92.1	35	10	28.6

TABLE 3

*Transmission of different strains of foreign P. vivax by A. quadrimaculatus*

ORIGIN OF STRAINS	MALARIA STRAINS TRANSMITTED TO:					
	White Patients		Negro Patients		Total Strains*	
	Attempts	Successes	Attempts	Successes	Attempts	Successes
Guadalcanal.....	15	11	6	3	17	11
New Guinea.....	11	11	5	1	11	11
Total Pacific.....	26	22	11	4	28	22
North Africa.....	6	5	2	1	8	6
Sicily & Italy.....	5	4	3	3	6	5
Total Mediterranean.....	11	9	5	4	14	11
Burma.....	1	1	0	0	1	1
Total.....	38	32	16	8	43	34

\* As some of the same strains were tried in both white and Negro patients, the totals are not necessarily the same as the addition of the numbers under these two categories.

New Guinea strains produced infections as did the one Burma strain. Fewer of the various strains infected Negroes than was the case with the white patients.

This, together with the total transmission rates shown in table 2, indicates that the Negro's resistance to *P. vivax* is not limited to strains from a particular area but is a general resistance to the species as found in widely separated regions.

*Comparison of the Transmission of Several Strains of Foreign P. vivax to Both White and Negro Neurosyphilitic Patients.* To study further the transmission

TABLE 4

*Comparison of Transmission of Several Strains of Foreign P. vivax to Both White and Negro Neurosyphilitic Patients*

STRAIN NUMBER	WHITE PATIENTS		NEGRO PATIENTS	
	Attempts	Successes	Attempts	Successes
1005G	6	6	1	0
1012G .	1	1	1	1
1019G	3	2	3	1
1023G	1	1	1	1
1017NG	1	1	1	0
1027NG	52	49	4	1
1030NG	4	4	2	0
1033NG	3	3	1	0
1034NG	2	2	1	0
1031Si	21	21	10*	2
1037Si	2	2	1	1
Total.....	96	92	26	7
Per Cent.....		95.8		26.9

\* One lot fed on Negroes but not on whites. Three patients involved—all failures.  
G—Guadalcanal; NG—New Guinea; Si—Sicily.

TABLE 5

*The effect of a previous infection of P. vivax malaria (St. Elizabeth strain) upon subsequent inoculation with foreign strains. White patients*

PATIENTS	P. VIVAX (ST. ELIZABETH STRAIN) NUMBER PAROXYSMS	FOREIGN P. VIVAX* NUMBER PAROXYSMS
J. M. D.	2	20†
A. C.	9‡	9
L. G.	13‡	11†

\* Three Pacific strains used—78, 90, and 94.

† Terminated by drug. All others self-terminated.

‡ Infection by blood inoculation. All others by mosquitoes.

rates to white and Negro patients, comparative tests were made using the same strains. With one exception, the same lot of infected mosquitoes was applied to both white and Negro patients at the same time. The number of infected mosquitoes biting each type of patient was about the same. These data are shown in table 4.

These results show the difference in susceptibility between Negroes and whites

to foreign *P. vivax* even more strikingly. The ratio of successful transmissions in white patients as compared with Negroes was 3.6 to 1.

*Effects of Previous Infections Upon the Development of Foreign P. vivax.* Three white neurosyphilitic patients who had just previously experienced a primary symptomatic infection of the St. Elizabeth strain of *P. vivax* were reinoculated by mosquitoes infected with foreign *P. vivax*. These reinoculations were given between 18 and 42 days after the last paroxysms with St. Elizabeth *P. vivax*. These data are presented in table 5.

Without treatment, the St. Elizabeth *P. vivax* infections had ceased to produce fevers and the parasite count had dropped to a low level indicating the production of an immunity against that strain. Such immunity did not prevent the foreign malarias from producing infections, which in 2 instances had to be terminated by drugs.

#### DISCUSSION

It appears from the data presented that white neurosyphilitics in this country will readily develop infections of foreign *P. vivax* when bitten by infected mosquitoes. The malarias originated from widely separated areas of the world and all showed a high rate of infectivity.

With Negro neurosyphilitic patients, the results were quite different. Of the total transmission attempts with all strains, the Negroes showed a much lower susceptibility. In a series in which the Negro and white patients were tested with the same strains, the difference was even greater, the Negroes being infected in only 26.9 per cent of the cases as against 95.8 per cent for the white patients.

It has been demonstrated repeatedly in this laboratory and by others (Boyd, M. F. and Stratman-Thomas, W. K., 1933) that *P. vivax* malaria usually cannot be induced in southern Negro neurosyphilitic patients, either by infected blood or mosquitoes.

Residence in a malarious area has been thought by some to be related to the resistance of Negroes to *vivax* infections. Among the Negro patients reported here, 29 were questioned as to previous residence. Five of 22 from the Southeast developed infections, including one with a previous malaria history. Four of the 7 from outside this area became infected. Even if these inadequate data were representative, there would still be a large unexplained difference between the two races.

The possibility of the presence of neurosyphilis affecting the susceptibility of Negroes to *P. vivax* malaria and not affecting white patients does not seem likely. Neurosyphilis does not seem to exert such a differential effect against *P. malariae* or *P. falciparum*.

Should the Negroes have a true racial immunity to foreign *P. vivax*, the possibility of the spread of these malarias in this country would be greatly lessened.

No great difference was found by us in the transmissibility of the *P. vivax* strain from widely separated areas, such as the South Pacific and Mediterranean. It appears that most of the imported foreign strains can be readily transmitted by *A. quadrimaculatus*.

## SUMMARY AND CONCLUSIONS

1. One or more attempts were made to transmit foreign *Plasmodium vivax* by *Anopheles quadrimaculatus* to each of 186 men. Of the 151 white patients, 94.7 per cent were infected. Of the 35 Negro patients, 31.4 percent were infected. On the first attempt, 92.1 per cent of the white patients and 28.6 per cent of the Negroes became infected.

2. When the same strains were tested against both, white patients were more readily infected than Negro patients in a ratio of 3.6 to 1.

3. The malarias tried originated from widely separated areas of the world and all showed a high rate of infectivity to white patients. The Negro seemed to have a general resistance to *P. vivax* from all areas rather than to strains from particular areas only.

4. In 3 white cases, a recent infection with the St. Elizabeth strain of *P. vivax* did not prevent the development of foreign strains of *P. vivax*. This indicated that little or no immunity was gained from the former strain. Should this be true for all American strains, the white population of this country could be considered as non-immune to foreign *P. vivax* malarias.

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# PENICILLIN THERAPY IN RELAPSING FEVER

## REPORT OF CASE

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A white soldier, aged 18 years, was admitted to the station hospital at Camp Bowie, Texas, August 16, 1944. He had been perfectly well until about a week before, when he began to experience slight but constant pain in the neck, elbows and knee joints. The night before he was hospitalized he had a shaking chill, followed by fever and headache, both of which had persisted. All other symptoms were denied.

Inquiry into his previous medical history revealed nothing of significance. He had served in the Merchant Marine, on runs to Glasgow, North Ireland and Murmansk, from August, 1943, to April, 1944. He was inducted into the Army in May, 1944, and when his illness began he had just returned from maneuvers, during which his company bivouacked beside the Colorado River and he had slept on a mattress cover filled with Spanish moss. He had no recollection of any insect bite during this period.

On admission to the hospital the patient was evidently acutely ill. The temperature was 103°F., the pulse rate 120, and the respiratory rate 20 per minute. Physical examination revealed no abnormality except injection and reddening of the pharynx and tonsils and a small follicular exudate over the left tonsil. Neurologic examination also revealed nothing abnormal.

The hemoglobin level was 13 gm. per cent. The white blood cell count was 12,800 per cubic millimeter. The differential count showed 91 per cent neutrophils, 8 per cent lymphocytes, and 1 per cent monocytes. On several other examinations the white blood cells varied between the level of the original examination and 8,300 per cubic millimeter. Urinalysis and the Kahn test were negative.

*Progress.* Chemotherapy with sulfadiazine was begun immediately on admission but was discontinued after 8 gm. had been given, when the tentative diagnosis of meningococcemia was ruled out.

On the second day of hospitalization the pharynx appeared more congested than on the first examination. Tiny patches of exudate were present over the uvula and both tonsils. Several cervical nodes were palpable bilaterally. Neither the liver nor the spleen could be felt.

Except for brief morning remissions to 101°F. the temperature was almost constantly elevated to 104°F. (fig. 1). The patient was delirious and on one occasion was found out of bed "looking for a friend on the wall." At the end of 48 hours the temperature fell to normal by crisis. Except for a few enlarged cervical and axillary lymph nodes physical examination at this time was essentially negative. The patient stated that he felt perfectly well except for mild muscular aches and low back pain.

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On the fourth day of hospitalization a transient pruritic rash was apparent over the entire body except the face; it consisted of uniformly distributed, pinkish, macular lesions, about 1 cm. in diameter, which cleared from the center.

On the fifth day (August 20) the patient had a chill, his temperature rose abruptly to 101°F. and a second febrile episode occurred similar in all respects to the first except that it lasted only 12 hours and that the rash was most marked on the chest, back, abdomen, and upper extremities. Similar episodes occurred August 25 and 29 and September 3. Physical examination, except for the transient rash following each episode, continued essentially negative.

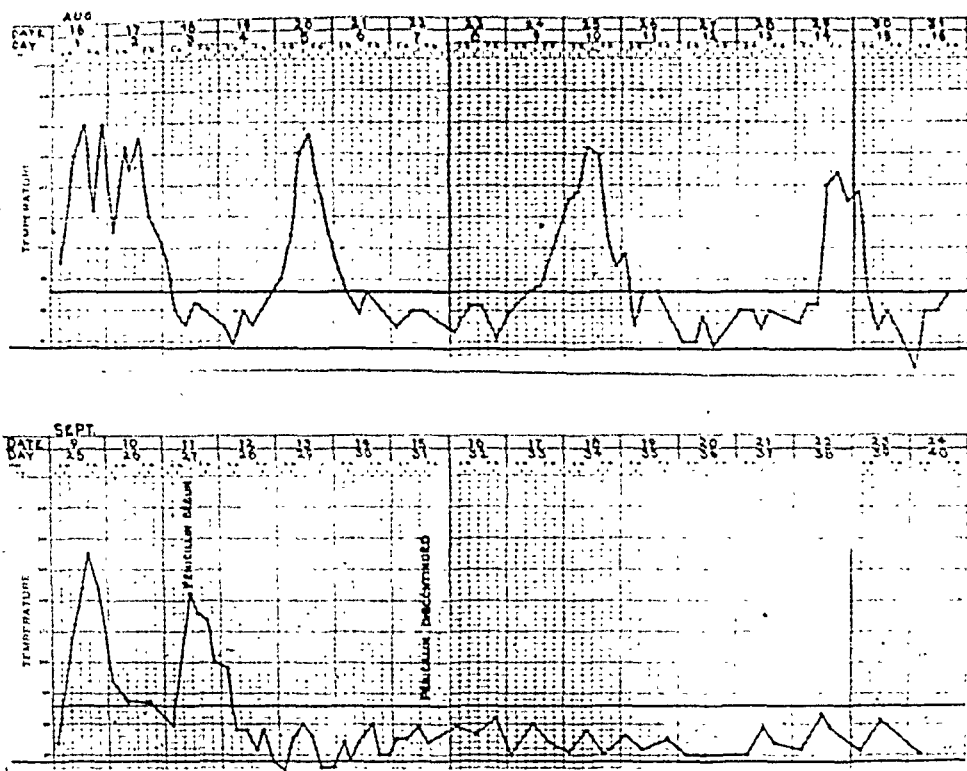


FIG. 1. TEMPERATURE CHART FOR FIRST 16 AND LAST 16 DAYS OF HOSPITALIZATION IN CASE OF RELAPSING FEVER TREATED BY PENICILLIN

*Diagnostic considerations.* Treatment had been chiefly symptomatic because no diagnosis had been made, although a number had been considered, including: Malaria, which was ruled out by failure to find plasmodia on repeated search with both thin and thick blood films.

Typhus fever, which was ruled out by the absence of characteristic mental symptoms and the lack of resemblance of the rash which occurred after each episode to the rash seen in typhus. In addition, the temperature elevation was not sustained and the Weil-Felix agglutination test was negative.

Infectious mononucleosis, which was ruled out by absence of atypical lymphocytes and by the negative heterophile antibody agglutination.

Meningococcemia, which was ruled out by negative blood cultures (under reduced oxygen tension as well as under aerobic conditions), the negative throat culture for *Neisseria intracellularis* (*N. meningitidis*) and the lack of response to chemotherapy.

Brucellosis, which was ruled out by the type of temperature curve and by negative agglutination titers.

Dengue fever, which was ruled out by the absence of splenic tenderness, of leukopenia, and of the characteristic "saddle back" temperature curve.

At a staff conference on the twenty-second day of hospitalization, the possibility of relapsing fever was first introduced, and in the next febrile phase, which occurred September 9, *Borrelia novyi* was demonstrated in blood films stained by Wright and Giemsa stains. A mouse was inoculated with the patient's blood, and within 60 hours spirochetes were demonstrated in its blood. *B. novyi* was also seen in fresh preparations examined by dark field illumination during the next febrile episode, which began September 11. During this episode moderate splenomegaly was observed for the first time.

*Further Progress.* Penicillin therapy was instituted coincident with the temperature elevation which began September 11. An initial dose of 60,000 units was given by continuous intravenous drip in 1,000 cc. of physiologic salt solution over a 6 hour period and was followed by the intramuscular injection of 20,000 unit doses at 3 hour intervals for the next 4 days. When treatment was discontinued a total of 650,000 units had been given.

The temperature returned to normal 12 hours after penicillin therapy was begun (fig. 1) and did not again rise. There was no recurrence of chills, muscular pains, rash, headache, or any other symptoms. When the patient was discharged September 24, 1944, 39 days after admission and 14 days after penicillin therapy was begun, he was apparently perfectly well.

He was observed at weekly intervals until November 21, 1944. Up to this time there had been no recurrence of symptoms or of temperature elevations, and he was returned to full military duty, on the presumption of cure.

#### COMMENT

Tick-borne relapsing fever appears as an endemic disease in the United States. It is confined entirely to the western and south-western states, and the majority of cases have occurred in Texas, where the first 3 cases were reported by Weller and Graham (1) in 1930. Like the case reported in this communication, they were contracted along the Colorado River, and it is of interest that Graham, while exploring the cave in which the tick bites occurred, himself contracted the disease. The tick vector, *Ornithodoros turicata*, is usually found in caves and overhanging ledges produced by erosion of the river banks. Most cases, as the one reported herewith, occur late in summer.

The American form of relapsing fever is ordinarily mild. It seems to be caused either by *Borrelia novyi*, as in this case, or by *Borrelia turicata*. Heilman and Herrell (2) have suggested that the many strains of the organism recovered in various parts of the world are all varieties of *Spirochaete obermeieri*.

The clinical picture and the clinical course in the case reported herewith followed the typical form of a sudden onset with chill, high fever, severe headache, muscular pain and abrupt crisis, followed by a quiescent interval terminated by repetition of the febrile episode. The rash was also typical. The pulse, however, was unusually slow. It reached 120 per minute only once, and even when the temperature was 104°F. it was often between 80 and 90. There was no evidence on the patient's body of any insect bite, and he specifically denied being bitten when he was questioned concerning the possibility. This is not unusual, the lesions often being so small and painless as to be undetected by the host when the bite occurs.

When the diagnosis of relapsing fever was finally introduced, it was confirmed without difficulty by staining methods, animal inoculation, and dark field illumination. The general opinion is that the Giemsa stain is several times more efficient in identifying the causative spirochete than any other diagnostic method, but in this case all the methods employed were successful.

Although arsenical therapy is usually stated to be specific in the treatment of relapsing fever, a review of the literature suggests that it is not entirely satisfactory. In some instances, usually of tick-borne disease, the infection has proved refractory, and relapsing fever, apparently louse-borne, has actually developed in the course of antisyphilitic therapy by an arsenical compound (3). Relapses and complications are not infrequent after arsenical therapy, particularly if it is begun at any time except at the onset of a febrile episode, although in Gillespie's (4) opinion there is a close correlation between relapse and inadequate dosage. Finally, arsenical-resistant strains of organisms may develop following arsenical therapy, and relapsing fever apparently makes the patient more susceptible to the ordinary hazards of arsenical compounds.

For these various reasons it is obvious that another form of therapy would be desirable in relapsing fever, and it was logical that penicillin should be tested for this purpose. Its effectiveness against the spirochetes of syphilis suggested that it might also be effective against the spirochetes of relapsing fever, in spite of fundamental differences in the metabolism of the two organisms.

A complete review of the literature is rather difficult at the present time because indices and journals arrive late, but as comprehensive a search as could be made to the date of writing (March, 1945) has revealed no previous clinical use of penicillin in relapsing fever. Several experimental investigations, however, have been conducted.

Heilman and Herrell (2) at the Mayo Clinic infected 54 mice with *Borrelia novyi*. Twenty-six animals were treated with a total of 4,000 Oxford units of sodium penicillin administered subcutaneously in 5 daily injections (1 of which, intended to last through the night, was in the amount of 500 units), over a 4 day period; the remaining 28 mice were used as controls. At the end of 24 hours the blood of the untreated mice showed severe infections while the blood of the treated mice showed a marked reduction in the number, and sometimes a total absence, of spirochetes. Twenty-one of the 28 control animals died, against only 1 of the treated mice. This particular animal had had no spirochetes in the blood

for several days, the spleen was not enlarged, and death did not occur until the eighth day after inoculation, though most of the untreated control mice were dead by the fourth day. All of the 7 surviving control mice suffered relapses but relapses occurred in only 4 of the treated mice. The dosage of penicillin used in this investigation was large, and no attempt was made to determine the minimum curative dose. The authors' conclusion was that penicillin therapy should eventually be possible in relapsing fever.

Lourie and Collier (5), using mice infected with *S. recurrentis*, found that the blood was usually cleared of the organism within 24 hours by the subcutaneous injection of 250 units of penicillin. The results were the same whether the drug was administered in a single injection or in 5 fractional doses of 50 units each at hourly intervals. Equally good results were achieved in control mice treated by the subcutaneous injection of 1 mg. of neoarsphenamine. The authors' conclusion was that penicillin is at least not inferior to arsenical therapy in relapsing fever and may prove to be even more effective, since the experimental dosage of penicillin used in the investigation was 26 times below the highest dose which mice could support without reaction, whereas the dosage of neoarsphenamine was only 4 times below the toxic level.

Augustine and his associates (6) treated 6 of 11 mice infected with *S. novyi* with 9,000 units each of penicillin given intraperitoneally over a 48 hour period; the other 5 mice were used as controls. Within 6 hours after treatment was begun the infections in the treated mice had decreased in intensity to about  $\frac{1}{4}$  but had increased about 50 per cent in the untreated animals. At the end of 26 hours no spirochetes could be demonstrated in the treated mice but organisms averaged 140 in single oil immersion fields in the untreated mice. Sixty hours after treatment was begun 2 of the apparently cured mice were sacrificed and citrated blood from the hearts was inoculated intraperitoneally into 2 fresh mice. No infections resulted. These authors concluded that on the basis of their preliminary experiments penicillin therapy is "spectacularly successful" in relapsing fever.

Eagle and his associates (7), who also experimentally infected white rats and mice with *Borrelia novyi*, found that approximately 400,000 units per kilogram of body weight was necessary to cure more than 95 per cent of the animals, this being about half the dose which killed a significant proportion. Their conclusion was that if these results can be translated to man, they would imply that the curative dose in human relapsing fever would be on the order of 25,000,000 units and that the therapeutic use of penicillin is therefore not warranted, except in arsenic-resistant cases, at least until the drug is available in larger quantities or unless relapsing fever is more amenable to treatment in man than it is in experimental animals.

While conclusions cannot be drawn from a single case, the immediate response to penicillin therapy in the reported case, and the apparent cure achieved by 650,000 units of the drug, suggest that the disease is more amenable in man than in animals. As Heilman and Herrell (2) pointed out, their own experimental results in mice infected with *Borrelia novyi* suggest that this strain is evidently

sensitive to the action of penicillin. Their further conclusion was that because of the close biologic similarity of other strains responsible for relapsing fever in other parts of the world, it seems probable that penicillin will also affect other strains of the organism. If this is true, and if the louse-borne disease becomes epidemic after this war, as it did after the last war, the apparent efficacy of penicillin therapy becomes extremely significant, for the death rate in several of the epidemics after the last war was very high.

#### SUMMARY

A case of tick-borne relapsing fever successfully treated by penicillin is reported. The experimental data concerning penicillin therapy in this disease are briefly reviewed and the possible clinical usefulness of this form of chemotherapy is pointed out.

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## A MILD EXANTHEMATOUS DISEASE SEEN IN THE SCHOUTEN ISLANDS

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During 1944 and 1945 a group of cases characterized by mild fever and constitutional symptoms, an exanthema of 7-10 days duration, joint pains and swellings enlarged lymph nodes and leucopenia was seen at the Schouten Islands in Geelvink Bay on the northern coast of Dutch New Guinea. Doctors at 3 hospitals independently recognized the condition as distinct and unusual and gave it various names. This report is based on 48 cases seen at two hospitals. At one hospital on Biak Island 31 cases from several different organizations were seen. From 2 to 9 patients per month were admitted during December 1944, April, May, June and July 1945. At another hospital on Owi Island, 17 cases were seen during March and April 1945. The first was a patient who had been transferred 2 weeks before he became ill from a hospital on Biak Island where he had had infectious hepatitis. His first symptoms began on March 18. Between March 20 and April 1, 8 members of the hospital staff were admitted with the disease. Simultaneously, 4 members of a small signal organization adjacent to the hospital developed the disease. Many of this group had had no intimate contact with patients. During the first half of April, 4 negro members of an engineering organization located further from the hospital than the signal company were afflicted. Because of the mild nature of this disease, these probably represent a small per cent of the cases that occurred.

### SYMPTOMATOLOGY

The subjective constitutional symptoms were mild (table 1). Headache in the frontal region, and backache or aches in various regions such as the shoulders, groins and legs were common. Post orbital pain and photophobia was noted in a small number of patients. Feverishness was a relatively common symptom but it was never very high. Only 2 cases had a definite chill though several complained of mild chilly sensations. Twenty-three per cent of the cases noted a dry cough, slight sore throat or symptoms suggestive of a beginning cold but these symptoms were only transient. Anorexia was a symptom in one quarter of the cases. One patient had sharp epigastric pain for three days before admission. He vomited once. The pain was worse when he was upright or rode in a car. There were no other complaints, but the characteristic rash was apparent at the time of admission on the 4th day of his illness.

More noticeable to the patient than any of the above symptoms was the rash. It was the appearance of this eruption that brought 45 or 94% of all cases to the hospital. Many cases were considered to have a skin disease and were referred to the out patient clinic for dermatological consultation. On admission 60%

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had no other complaint than rash. Accompanying the rash 25% of the cases complained of itching, usually generalized, but often most intense on the palms or soles especially if there was swelling of these sites. A very characteristic complaint was pain and swelling of the ears, noted by 40% of the patients. This was limited to the edge of the auricle. It was occasionally the first symptom. In addition swelling of the eyelids was common.

Stiffness and tenderness of a joint was a complaint in 14% of this series. The joint involvement was never severe enough to prevent the patient from walking into the hospital.

Physical examination on admission to the hospital showed the patients did not appear acutely ill, nor in the least prostrated. They were comfortable in bed

TABLE 1  
*Symptomatology*

	<i>Per cent of cases</i>
<b>Constitutional manifestations</b>	
Fever of 100° or over.....	75%
Headache.....	50%
Backache and generalized muscle aches.....	40%
Anorexia.....	23%
Postorbital pain.....	17%
Photophobia.....	8%
Chill.....	4%
<b>Maculo-papular rash</b>	
Swelling of ears.....	100%
Swelling of skin over elbows and knees.....	46%
Swelling of hands and feet.....	46%
Swelling of eyelids.....	25%
Lesions on oral mucous membranes.....	14%
<b>Joint involvement</b>	
Effusion.....	31%
Lymph node enlargement.....	10%
Splenomegaly.....	80%
<b>Leucopenia</b>	
W.B.C. 5000 or less.....	10%
W.B.C. 4000 or less.....	39%

alert, and cheerful. Mild analgesics were sufficient to relieve the aches. The symptoms were not aggravated by activity.

The rash was so characteristic that it immediately established the diagnosis. It appeared on the first day of illness in 28 cases (58%). In the first 3 days of the disease the rash had appeared in 85%. In 4 cases it did not appear until the 4th day, in 2 cases on the 5th day and one case was ill 6 days before the rash appeared (table 2.). It began as a macular erythematous eruption, appearing first over the thorax, anteriorly and laterally, then spreading to cover the chest, back, shoulders, upper arms more on the extensor than the flexor surfaces, and then progressively down the arms, trunk and lower extremities. Sometimes it involved the neck and face as well. The area of the lower abdomen, buttocks and

upper thighs often showed fewer lesions than sites mentioned. Soon after their appearance, the macules began to enlarge to a diameter of 10 mm. or more. Then the whole lesion either became slightly elevated and dusky red, or more commonly a small papule developed in the center of the macule and both macule and papule enlarged. Sometimes there would be 2 to 5 papules of varying sizes in one macular lesion. In these instances the underlying macule tended to remain flat and bright red until the defervescence when the dusky red color would appear. In the more florid cases the papules enlarged until they became 2 to 3 mm. in diameter and 1 to 3 mm. in height. At this stage they would be pale on top and superficially resemble vesicles. At the height of the eruption the underlying macules became confluent, covering large areas of the skin. Every case in this series showed this type of rash in various degrees of severity and extent.

In 46% of the cases the exanthem involved the ears with redness and swelling of the auricle along the helix and antihelix. Several cases showed redness and swelling of the tip and alae of the nose in addition. The palms of the hands and soles of the feet were included in the eruption in 25%. The macules in these cases were always bright red. They were uniformly distributed on the palms and were usually grouped along the lateral margin of the foot or the medial side of the instep. In the majority of cases these lesions were accompanied by swelling of the underlying tissues and a moderately severe tenderness to pressure. In some cases the swelling was so severe that the whole palm was puffed. Stiffness and pain were then such that motion of the fingers was very uncomfortable. In these instances itching also was intense. A very characteristic localization of the rash was over the elbows and on the anterior aspect of the lower thigh in the prepatellar region. In these sites the lesions were bright red and the involved skin was thickened by an edematous induration. These lesions frequently coalesced, leaving the skin over the olecranon processes and more often over both knees fiery red and tender similar to an area of erysipelas. There were 7 cases in which swelling of the eyelids was noted. This appeared as a periorbital puffiness which was occasionally sufficient to markedly narrow the palpebral fissure. Sometimes it was so mild as to be overlooked until the contrast caused by its sudden disappearance would call it to attention. Lesions on the mucous membrane of the palate were noted in 7 cases. In all of these the skin eruption was very marked and lasted 10 days or more. They were small bright red macular areas 2 to 4 mm. in diameter scattered along the hard palate or at its junction with the soft palate. There was no resemblance to Koplik spots. These macules disappeared shortly after the exanthem began to fade.

After the height of the rash was reached there was a sudden and dramatic change. The color would darken overnight and the lesions became more discrete. The papules subsided rapidly and the macules would no longer completely blanch on pressure. After a few days the macules appeared as faint brownish discolored areas. Occasionally the lesions took on a purplish red color as they faded. In a few cases they became definitely purpuric, especially on the palms and soles. No cases were noted in which there was any desquamation. The eruption disappeared from the fourth to the fourteenth day of the disease. As



shown in table 2, the majority had the rash from 5 to 10 days. The average duration was 7.5 days.

Enlargement of the lymph nodes was conspicuous. It was present in 80% of this series. The axillary, inguinal, femoral, and epitrochlear nodes were consistently involved. Most cases had enlarged cervical nodes as well. There were a few cases however in which the cervical nodes were the only ones noted. The glands were discrete, firm, rubbery, about 1 to 2 cm. in diameter and not uncommonly tender. There was no apparent correlation between the extent of the rash and the degree of lymph node enlargement.

Another of the distinctive features was involvement of the joints. It was much more prominent in this disease than in any other exanthemata, occurring in 31% of the group. The patients complained of stiffness and soreness and there was moderate swelling about the joint. A small amount of fluid accumulated in the joint spaces in approximately half of these cases. Motion was only slightly limited due to pressure or stiffness. There was mild tenderness on palpation.

The knee was involved in 6 cases, and of these 3 had fluid in both knee joints as demonstrated by the finding of a patellar tap. The other joints involved were

TABLE 2  
*Relation of the rash to the febrile stage*

Day of disease.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Duration of fever														
Number of cases.....	4	11	17	7	3	4	1							
Onset of rash														
Number of cases.....	28	3	10	4	2	1								
Termination of rash														
Number of cases.....				5	8	5	6	5	7	6	2	2	1	1

the ankle, (4 cases) a metacarpophalangeal joint (2 cases), and the hip, elbow and wrist. One other case showed swelling over the styloid process of the left ulna. The swellings appeared as early as the 3rd day of the disease and as late as the 9th day but most cases developed from the 5th to the 7th day. The onset usually coincided with the height of the rash or appeared the day the rash began to fade. The duration of joint involvement depended on the degree. Mild swelling would subside in one or two days, while effusions required 4 to 5 days. Two cases had fluid in the knee joint for 9 days. The symptoms rapidly disappeared as the swelling subsided. No evidence appeared to indicate any serious damage to the synovial surfaces and there was no recurrence of swelling, pain or limitation of motion after recovery.

Splenomegaly was a relatively infrequent finding. It occurred in only 5 cases. The liver was palpable in 3 cases, and became enlarged during the course of the disease in one of these. Neither organ was noted to be tender on examination.

The temperature course in this disease was very mild. The fever lasted from 1 to 7 days but 66% of the cases had an elevation for 3 days or less (table 2). The degree of elevation was moderate or slight. The highest recorded was 102.8 on one

occasion. Seventy-seven per cent of the cases never had a temperature over 101°F. The usual course was of prompt fall of the fever curve to normal by the second or third day in the hospital. From that time on the temperature remained normal in all cases except one patient who had an irregular slight fever for 6 days.

In other respects the course of the illness was entirely favorable. The appetite and sense of well being returned promptly after the febrile response was over, and before the rash began to fade. As soon as the rash disappeared the patients were completely recovered. There was no asthenia nor mental depression during convalescence.

#### LABORATORY DATA

The data from laboratory examinations done on 31 patients showed the presence of a leucopenia was consistent. Twelve cases had white blood cell counts below 4,000 per cu. mm. and in 25 cases the white cells numbered less than 5,000 per cu. mm. (table 1). Some cases (30%) showed a moderate shift to the left and others (22%) showed a relative lymphocytosis of 45% or more. Blood cultures were sterile in 15 cases. One patient showed one blood culture positive for *Salmonella schottmulleri*. Another case had 3 blood cultures which were positive for *Streptococcus viridans*. Three subsequent cultures were sterile. There were no petechiae observed, the patient had no fever, and the heart was normal on physical examination. The significance of this finding was never explained, but seemed to have no bearing on the disease under discussion. Urine cultures were negative in 13 cases and showed *Salmonella schottmulleri* in two. Stool cultures revealed no pathogenic organisms in 11 instances. One case had *Salmonella schottmulleri* in the feces and 2 cases showed *Shigella paradysenteriae* (Flexner) bacillus. Neither of the latter had any diarrhea and the organisms were not found on repeated cultures.

There is no adequate explanation for all the instances in which *Salmonella* and *Shigella* organisms were obtained on culture. Carriers of these pathogens were common in this locality and some of these results could be explained on this basis. This would not explain the positive blood and urine cultures. In view of the fact that the majority of cultures did not show this or any other organism it is felt that this finding is not related to the etiology of this disease.

Darkfield examinations of the blood in 8 cases and of the skin lesions in one case failed to reveal spirochetes or leptospira. Agglutination tests were negative. These included OXK, Widal (Typhoid O and H, Para A and B), Heterophile antibodies, and one strain of Brucella. In none of these tests was the titre higher than the accepted normal range. These tests were done both early and late in the course of the disease. The Kahn was negative for all cases.

#### DIFFERENTIAL DIAGNOSIS

The appearance of the rash resembled measles. However it did not start on the face and in most cases spared this region. In addition there were no Koplik spots nor coryza and the constitutional symptoms usually subsided as the rash developed. Twelve cases gave a definite history of having had measles, one only



# INTESTINAL HELMINTHIASIS: CLINICAL SURVEY OF SIX HUNDRED AND EIGHTEEN CASES OF INFECTION WITH COMMON INTESTINAL HELMINTHS IN CHILDREN

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One cannot practice pediatrics in the tropics without being impressed by the frequency of infection with intestinal parasites. These infections are widely prevalent on the Isthmus of Panama where instead of being chiefly of academic interest as they are in most of the United States, they are of prime clinical importance.

Excellent contributions to the knowledge of helminthiasis have appeared in the literature. These detailed accounts, however, have been devoted more to biologic than to clinical aspects, particularly as the latter relate to children. The discussions devoted to symptoms, physical findings and treatment have been brief, confusing, and frequently not borne out by observation. These existing misconceptions concerning the incidence, clinical manifestations and treatment of intestinal helminthiasis in children prompted this survey.

## SOURCE OF MATERIAL

This study deals with 518 children infected with intestinal parasites who were admitted to the pediatric wards of Gorgas Hospital, Ancon, Canal Zone from January 1, 1941 to January 1, 1944. An additional 100 outpatients infected with *Enterobius vermicularis* were included in this survey making a grand total of 618. They were divided as follows: Group 1, consisting of 457 patients in whom infection was limited to a single parasite, and group 2 consisting of 161 patients infected by 2 or more parasites (table 1). Separate communications dealing with oxyuriasis (1), ascariasis (2), trichuriasis (3), ancylostomiasis and strongyloidiasis (4), and intestinal polyparasitism (5) have been published. This presentation is a summary of the clinical data obtained in the complete survey.

The common parasitic infections in children were found to be ascariasis, ancylostomiasis, trichuriasis, oxyuriasis and strongyloidiasis. Some patients were infected with 2 or more parasites so that the frequency of infection in 518 parasitized patients treated in the hospital was as follows:

PARASITIC INFECTION	NUMBER	PER CENT
Ascariasis.....	247	33.7
Ancylostomiasis.....	175	23.8
Trichuriasis.....	153	20.8
Oxyuriasis.....	115	15.7
Strongyloidiasis.....	44	5.9
Total.....	734	

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The patients were children of employees of United States governmental agencies on the Isthmus of Panama. No socioeconomic selection of patients existed in this series and all races, groups and social levels were represented.

TABLE 1

*Distribution of intestinal parasites in children in a large general hospital located on the Isthmus of Panama*

The 200 patients with oxyuriasis included 100 treated in the hospital and 100 treated in the outpatient clinic.

	NUMBER OF CASES	WHITE		NEGRO		MESTIZO*	
		No.	%	No.	%	No.	%
Group 1							
Ascariasis.....	125	7	5.6	46	36.8	72	57.6
Oxyuriasis.....	200	161	80.5	20	10.0	19	9.5
Ancylostomiasis.....	71	4	5.6	33	46.4	34	48.0
Trichuriasis.....	50	3	6.0	31	62.0	16	32.0
Strongyloidiasis.....	11	1	9.0	8	73.0	2	18.0
Group 2							
Ascariasis and trichuriasis.....	42	1	2.4	11	26.2	30	71.4
Ascariasis and ancylostomiasis.....	34	0	0.0	1	2.9	33	97.1
Ascariasis, ancylostomiasis, and trichuriasis.....	22	0	0.0	2	9.0	20	91.0
Ascariasis, ancylostomiasis, strongyloidiasis and trichuriasis.....	9	0	0.0	1	11.1	8	88.9
Ascariasis and oxyuriasis.....	6	1	16.7	1	16.7	4	66.6
Ascariasis, ancylostomiasis and strongyloidiasis.....	3	0	0.0	1	33.3	2	66.7
Ascariasis and strongyloidiasis.....	2	0	0.0	0	0.0	2	100.0
Ascariasis, oxyuriasis and trichuriasis.....	2	0	0.0	0	0.0	2	100.0
Ascariasis, strongyloidiasis and trichuriasis.....	2	0	0.0	0	0.0	2	100.0
Ancylostomiasis and trichuriasis.....	16	0	0.0	5	31.3	11	68.7
Ancylostomiasis and strongyloidiasis.....	9	0	0.0	2	22.2	7	77.8
Ancylostomiasis, strongyloidiasis and trichuriasis.....	7	0	0.0	3	42.8	4	57.2
Ancylostomiasis and oxyuriasis.....	3	0	0.0	1	33.3	2	66.7
Ancylostomiasis, oxyuriasis and trichuriasis.....	1	0	0.0	0	0.0	1	100.0
Oxyuriasis and trichuriasis.....	2	0	0.0	0	0.0	2	100.0
Oxyuriasis and strongyloidiasis.....	1	0	0.0	1	100.0	0	0.0
Total.....	618	178	28.8	167	27.0	273	44.2

\* The term mestizo is used to indicate various mixtures of Indian, Negro, white and oriental stocks which constitute the bulk of the native population.

#### CLINICAL CONSIDERATIONS

*Location of dwellings*—The communities from which the patients came were of three types as follows:

1. United States government communities located in the Canal Zone, char-

acterized by minimal crowding, excellent toilet and sewage disposal facilities, paved streets and landscaped yards.

2. Principal cities of the Republic of Panama. In the center of these cities overcrowding among the poor is commonplace, facilities for sewage disposal are adequate, although not used to the fullest extent by an uninformed populace, and streets and alleys are paved. In the outskirts of these cities, however, facilities for sewage disposal are minimal; streets unpaved and landscaping absent.

3. Rural or semirural communities in the Canal Zone or Republic of Panama (interior) where population is sparse, living conditions primitive, even outhouses being seen infrequently.

In order to determine whether type of location of dwellings had any casual relationship to helminthiasis, the available data from this series of 618 patients were analyzed (table 2). It was obvious that oxyuriasis occurred most fre-

TABLE 2

*Residencies of patients infected with intestinal parasites*

These figures were obtained from statistical data on the admission records of the patients. Subsequent questioning of parents revealed that the percentage dwelling in large cities of the Republic of Panama was too high and the number dwelling in unsanitated rural areas was too low.

PARASITIC INFECTION	LOCATION OF DWELLINGS		
	Government communities in Canal Zone	Large cities in Republic of Panama	Unsanitated rural areas
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Ancylostomiasis.....	7.0	17.0	76.0
Ascariasis.....	12.0	26.4	61.6
Oxyuriasis.....	82.5	13.5	4.0
Strongyloidiasis.....	27.0	27.0	46.0
Trichuriasis.....	6.0	44.0	50.0
Polyparasitism.....	6.8	21.1	72.1

quently in areas where personal hygiene, living and environmental conditions were at a high level. Ancylostomiasis, ascariasis, and trichuriasis, on the other hand, were infections which occurred predominantly in areas where these factors were at a low level and combined to favor embryonation of ova in polluted soil. The 11 children infected with *Strongyloides stercoralis* made up too small a group to permit generalization. That a total of 104 or 24.9 per cent of the 418 patients with ancylostomiasis, ascariasis, strongyloidiasis, trichuriasis and polyparasitism came from large cities in the Republic of Panama was contrary to expectations. These figures obviously were misleading because such infections should be uncommon in the city with sanitation facilities. Further investigation revealed that most of these patients acquired their infections in rural districts and were brought into the cities by parents employed by agencies of the United States government.

*Racial differences*—Important differences in the incidence of intestinal para-

sites among white, mestizo and Negro children were observed (table 1). Except in strongyloidiasis, where the number of patients was too small to be significant, and in oxyuriasis where the white child was affected most frequently, the highest incidence was in the mestizo, and the lowest in the white child. The incidence among Negroes occupied an intermediate position. Except for oxyuriasis this distribution was considered as being a logical result of living conditions and not due to racial susceptibility.

In general, the white children lived where sanitation, hygiene and environmental factors were entirely satisfactory, the Negroes lived where these factors were only fairly satisfactory, and the mestizos actually lived on the soil. It was

TABLE 3

*Age distribution of patients infected with intestinal parasites (in per cent)*

PARASITIC INFECTION	AGE IN YEARS												
	Less than 1 yr.	1	2	3	4	5	6	7	8	9	10	11	12
Ancylostomiasis...	0.0	2.8	2.8	0.0	7.0	4.2	22.5	11.3	7.0	16.9	7.0	9.9	8.5
Ascariasis.....	0.8	16.0	12.0	16.8	7.2	12.0	7.2	3.2	2.4	3.2	7.2	4.8	7.2
Oxyuriasis.....	0.0	5.5	15.0	13.5	13.0	11.5	7.0	6.5	7.0	9.0	5.5	2.5	4.0
Strongyloidiasis...	0.0	27.2	9.0	18.0	0.0	9.0	0.0	0.0	18.0	0.0	9.0	9.0	0.0
Trichuriasis.....	0.0	12.0	2.0	12.0	12.0	8.0	10.0	2.0	12.0	12.0	4.0	8.0	6.0
Polyparasitism....	0.6	3.1	13.7	7.5	12.4	11.8	11.8	8.1	5.0	7.5	5.6	6.2	6.8

TABLE 4

*Distribution of intestinal parasites according to sex of patients*

PARASITIC INFECTION	PER CENT OF PATIENTS	
	Male	Female
Ancylostomiasis.....	64.8	35.2
Ascariasis.....	54.4	45.6
Oxyuriasis.....	50.0	50.0
Strongyloidiasis.....	45.4	54.6
Trichuriasis.....	56.0	44.0
Polyparasitism.....	64.0	36.0

not surprising, therefore, to find the highest incidence of intestinal parasitism in the mestizos, and the lowest in white children. The observation that oxyuriasis was much more common in white than in Negro children confirmed a similar observation made by Cram (6) in studies conducted in Washington, D. C.

*Age*—The age distribution is shown in table 3. Except in infancy oxyuriasis was more or less evenly distributed throughout the preschool age. From then on there was a gradual fall continuing probably into adult life.

Ascariasis was observed in an 11 month girl who died from a heavy infection and resultant complications. In this child infection must have occurred at or before the age of 9 months. Infection with *Ascaris lumbricoides* might occur as soon as

a child was old enough to crawl about and come in contact with polluted soil. In 52.8 per cent of the patients, infection occurred by the time the patients were 4 years of age. The younger children not only became infected more commonly than older children, but they also harbored heavier infections and showed more serious symptoms and physical signs. The incidence of trichuriasis in children of the preschool age corresponded to that of ascariasis. From then on, the incidence of infection with *A. lumbricoides* decreased as the habits of patients improved and the parasites died spontaneously. However, the incidence of trichuriasis was maintained throughout childhood probably because of the longer life span of *Trichuris trichiura*. Ancylostomiasis was the least common infection during the preschool age, it having been discovered by the age of 4 years in only 12.6 per cent of the patients.

Polyparasitism was uncommon before the age of 2 years. The incidence then increased rapidly up to the age of 5 when 79 or approximately one-half of the children had accumulated their burden of worms.

*Sex*—There was a predominance of boys in every infection considered except in oxyuriasis and strongyloidiasis (table 4).

#### SYMPTOMATOLOGY OF HELMINTHIASIS

Of the 618 patients studied, 367 or 59.4 per cent were brought to the physician for diagnosis and treatment of diseases other than parasitism, and 251 or 40.6 per cent with symptoms later accounted for by helminthiasis alone (table 5). Malaria and miscellaneous conditions of the respiratory tract were the most frequent unrelated associated diseases observed. It has been pointed out that oxyuriasis occurred commonly in the white child who lived in the sanitated community, and, therefore, one would not expect to find malaria in children infected with *E. vermicularis*. Such proved to be the case in this study. Only 1 per cent of the 200 patients with oxyuriasis had malaria. Of the remaining 418 with ancylostomiasis, ascariasis, strongyloidiasis, trichuriasis and polyparasitism 195 or 46.7 per cent had malaria.

A liberal attitude was maintained in evaluating the symptomatology of intestinal parasitism. Symptoms which could have been due to either helminthiasis or to associated unrelated disease were included with those presumably due to parasitism. Such symptoms as chills and fever, delirium, headache, purulent aural discharge, rhinorrhea, skin rash, somatic pains, and sore throat obviously due to associated unrelated diseases were discarded completely. By these procedures it was hoped to obtain an accurate index of the symptomatology of helminthiasis.

All symptoms observed were placed arbitrarily into the following groups of symptoms: (1) General; (2) local; (3) those secondary to local discomfort; (4) gastrointestinal; (5) nervous; and (6) pulmonary (table 6). This classification was adopted because it simplified understanding the clinical manifestations. The actual placement of findings in one group or another was a convenience arbitrarily adopted for purposes of this report.



*Symptomatology of oxyuriasis*—One hundred hospital patients and 100 outpatients infected with *E. vermicularis* were studied. Most of the former entered the hospital because of diseases unrelated to oxyuriasis, while most of the latter had no disease other than oxyuriasis. Seventy-five of the inpatients and 50 of the outpatients were free from subjective symptoms attributable to oxyuriasis. Thus, in 125 or 62.5 per cent, the presence of infection was determined by laboratory means or by the mother or nurse seeing the worms on the perianal region.

The following symptoms were observed:

A. Local symptoms: (1) pruritis ani and associated sensations; and (2) vaginal and perianal irritations with or without secondary infection, due mostly to scratching.

B. Symptoms secondary to local discomfort: (1) Wakefulness at night; (2) genitourinary disturbances such as nocturia, burning on urination, and enuresis or any other symptoms occasioned by nonspecific vaginitis.

TABLE 5  
*Associated unrelated diseases in intestinal parasitism*

DISEASE	PER CENT OF PATIENTS					
	Ancylostomiasis	Ascariasis	Oxyuriasis	Strongyloidiasis	Trichuriasis	Polyparasitism
Malaria.....	69.0	55.2	1.0	45.5	22.0	37.9
Acute and chronic respiratory diseases.....	14.1	16.0	34.0	27.3	30.0	21.2
Acute communicable diseases..	5.6	4.8	3.0	0.0	6.0	6.2
Miscellaneous.....	11.3	3.2	12.5	9.0	20.0	8.7
Total.....	100.0	79.2	50.5	81.8	78.0	74.0
Intestinal parasitism unassociated with other diseases..	0.0	20.8	49.5	18.2	22.0	26.0

C. Gastrointestinal symptoms; (1) Abdominal pain; (2) vomiting; (3) diarrhea; and (4) anorexia.

D. Nervous symptoms secondary to disturbed sleep and general discomfort. Although no specific disorders were observed, it was obvious that behavior or personality problems could be caused or aggravated by severe perianal itching. Fretfulness, gritting of the teeth, headaches, irritability, lethargy, restlessness, thumb sucking and tics were observed occasionally, although these symptoms did not include all of the possibilities.

Anemia, malnutrition of marked degree and allergic manifestations were not encountered. Convulsions were present in 2 patients, both of whom had fever and acute suppurative otitis media.

*Symptomatology of ascariasis*—The symptoms of ascariasis are produced by (1) migrating larvae, and (2) by adult worms in the gastrointestinal tract or its associated organs. The symptoms associated with migration of larvae are those produced by lodgement of larvae in various organs of the body. Larvae have

TABLE 6

*Symptomatology of oxyuriasis, ascariasis, trichuriasis and polyparasitism*

INFECTION	PER CENT OF PATIENTS			
	Ascariasis	Oxyuriasis	Trichuriasis	Polyparasitism
Number of cases.....	125	200	50	161
General symptoms				
Failing health.....				0.6
Failure to gain or loss of weight.....	6.4	3.5	10.0	1.9
Fever.....	11.2		6.0	19.3
Geophagy.....			2.0	
Malaise.....			2.0	
Malnutrition.....	52.8			39.1
Local symptoms				
Choking.....	0.8			
Frequency of urination.....	0.8			
Itching in throat.....	0.8			
Pruritis ani.....		8.5	2.0	
Vaginitis, local infections.....		1.5		
Worms on perianal region.....		36.0		
Symptoms secondary to local discomfort				
Burning, enuresis, nocturia.....		2.5		
Wakefulness at night.....		4.0		
Gastrointestinal symptoms				
Abdominal distention.....	1.6			4.3
Abdominal pain.....	8.0	12.0	18.0	13.0
Anorexia.....	8.8	6.5	4.0	14.9
Bloody diarrhea.....	1.6		10.0	4.3
Constipation.....			2.0	0.6
Diarrhea.....	8.8*	1.5	14.0*	15.5*
Excessive appetite.....	0.8			
Passing worms by bowel.....	22.4†		14.0	13.0†
Passing worms by mouth & bowel.....	3.1			3.1
Prolapse of rectum.....			4.0	2.5
Tenesmus.....			2.0	2.5
Vomiting.....	22.4†	4.5	10.0	19.9†
Vomiting worms.....	10.4†			6.2†
Nervous symptoms				
Apathy, lethargy, weakness.....	2.4	1.5	4.0	4.3
Convulsions.....	4.8	1.0		1.9
Facial tics, grimacing.....		1.0		
Fretfulness, irritability, restlessness.....	3.2	3.0		
Gritting teeth.....		0.5		
Headache.....		0.5		1.9
Thumb sucking.....		0.5		
Pulmonary symptoms				
Cough.....	5.6			13.0
Dyspnea.....				2.5

\* Includes bloody diarrhea.

† Includes passing of worms by mouth and bowel.

‡ Includes vomiting of worms as well as passing of worms by mouth and bowel.

been reported in the brain, kidneys, lungs, spinal cord, spleen and thyroid gland. Autopsies were performed on 5 patients in the present study, and although the internal organs were examined carefully both grossly and microscopically, larvae were demonstrated in only 1 patient (in the middle ears). The presence of larvae in the lungs is said to result in fever, rapid respiration, hemoptysis and signs of consolidation. Lobar or bronchopneumonia was diagnosed in 5 patients who had ascariasis, and in 9 others who had ascariasis in combination with other helminthic infections. It is not asserted, however, that the pneumonias in these cases were due to the presence of larval forms in the lungs or were of the atypical lobular type called by Keller and his associates (7) *Ascaris pneumonitis*. Except in the 14 children with pneumonia, abnormal physical signs in the chest were observed rarely, and roentgenographic examinations of the lungs of 30 additional patients who had findings implicating the respiratory tract revealed essentially normal conditions. Sputum was examined in a few instances, but no larvae were demonstrated. In many of the patients, infection was continuous so that combinations of symptoms due to migrating larvae and to adult forms were observed. Of the 14 who had pneumonia, 5 passed ascarides in their stools, and 1 vomited as well as passed worms in his stools.

The following symptoms were observed in 125 patients infected with *A. lumbricoides*:

A. General symptoms: (1) Malnutrition; (2) fever; and (3) loss of or failure to gain weight. Although quantitative estimation of the exact state of nutrition in these children was not possible, 66 or 52.8 per cent were described as being underweight. This finding was most pronounced in the younger patients.

B. Local symptoms: (1) Choking; (2) frequency of urination; and (3) itching in throat.

C. Gastrointestinal symptoms: (1) Passing of worms by bowel; (2) vomiting; (3) vomiting of worms; (4) passing of worms by mouth and bowel; (5) anorexia; (6) abdominal pain; (7) diarrhea; (8) abdominal distention; (9) bloody diarrhea and (10) excessive appetite. The two most frequent symptoms were passing of worms by bowel and vomiting. The former occurred in 28 patients (22.4 per cent), 18 of whom were brought to the hospital for unrelated disease, the history of passing worms being obtained incidentally. Vomiting was of almost equal importance as a symptom; it occurred in 28 patients, or 22.4 per cent, of whom 13, or 10.4 per cent, vomited worms. Included in this total were 4 patients who vomited worms as well as passed them by bowel. Abdominal complaints were more often in the nature of abdominal distress rather than actual pain. Acute disturbances due to lodgement of parasites in the gastrointestinal tract or its associated organs have been reported. Ascarides may produce intestinal obstruction. They may wander into the appendix producing obstruction of the lumen and symptoms of appendicitis; into the common bile duct producing jaundice and/or suppurative pancreatitis, or rupture the wall of the appendix or intestine resulting in peritonitis. Fatal abdominal accidents were observed twice. In 1 child, several ascarides entered the peritoneal cavity through a perforation in the appendix and resulted in generalized peritonitis. In the other child, the liver, biliary and pancreatic ducts were invaded by the parasites.

D. Nervous symptoms: (1) Convulsions; (2) restlessness, (3) apathy and weakness. Convulsions were observed in 6 patients: 4 had no disease other than ascariasis, 1 had an associated acute bilateral suppurative otitis media, and 1 had an associated primary estivoautumnal malaria. These acute infections offered more tenable explanations than did ascariasis, although it could not be stated with finality that either the unrelated associated diseases or ascariasis were the cause. Apathy, irritability, restlessness and weakness were uncommon complaints.

E. Pulmonary symptoms: (1) Cough.

*Symptomatology of ancylostomiasis*—Relatively few symptoms were observed in the 71 patients with ancylostomiasis. Abdominal pain in 1 patient and vomiting in 2 others were thought to be due to this infection. Four children, 1 in whom ancylostomiasis was the sole infection and 3 in whom ancylostomiasis was part of a multiple parasitic infection showed severe anemia, pallor, cardiac dilatation and failure. Even in the presence of severe anemia, subjective symptoms were not observed until the anemia reached such a degree that critical heart failure supervened. Malnutrition, retardation in growth and development, and abdominal distention were uncommon except in severe infections.

*Symptomatology of trichuriasis*—The symptoms observed in 50 patients with trichuriasis were as follows:

A. General symptoms: (1) Failure to gain or loss of weight; (2) fever; (3) geophagy; and (4) malaise.

B. Local symptoms: (1) Pruritis ani.

C. Gastrointestinal symptoms: (1) Abdominal pain; (2) diarrhea, oftentimes blood-streaked; (3) passing of worms by bowel; (4) vomiting; (5) anorexia; (6) tenesmus; (7) prolapse of the rectum; and (8) constipation.

The most common complaint was abdominal pain or distress. In only 1 instance was the pain localized in the right lower quadrant thereby simulating appendicitis. There were several children under the age of 2 years in whom the diarrhea was associated with marked dehydration, loss of weight and loss of tissue elasticity. Generally, however, the diarrhea of trichuriasis extended over a period of one to three months. The stools numbered 4 to 6 daily, were loose but usually not watery, and frequently were streaked with blood. Prolapse of the rectum occurred in 2 patients infected solely with *T. trichiura*, and in 4 patients in whom this parasite occurred in combination with other helminths. Six patients had a blood-streaked diarrhea, and in several with prolapse, parents reported noticing small white worms on the surface of the prolapsed mucosa. Although diarrhea was observed in 53 or 8.6 per cent of the 618 patients in this survey, rectal prolapse occurred only in those who had ova of *T. trichiura* in their stools. It was felt that a typical history of severe trichuriasis in the infant or young child was as follows: (1) diarrhea of from one to three months duration, the stools often becoming streaked with blood as the disease progressed; (2) recurring abdominal discomfort and tenesmus; (3) progressive loss of weight eventuating in (4) repeated prolapse of the rectum.

D. Nervous symptoms: (1) Apathy and weakness.

*Symptomatology of strongyloidiasis*—Symptoms of infection with *S. stercoralis*

were common even in this small group of 11 patients. Vomiting occurred in 2 children, diarrhea in 2, loss of weight, abdominal tenderness, and lethargy in 1 each. Both patients with diarrhea had intermittent attacks of vomiting and loose stools extending over a period of several months without fever or associated disease. Other causes of diarrhea with blood and mucus in the stools were ruled out. None had stools that were grossly streaked with blood as were observed in trichuriasis. Nutrition of the group was average.

*Symptomatology of polyparasitism*—The symptoms observed in 161 patients infected with 2 or more intestinal parasites were as follows:

A. General symptoms: (1) Malnutrition or underweight; (2) fever; (3) loss of weight; (4) failing health and loss of desire to play.

B. Local symptoms: (1) Pruritis ani.

C. Gastrointestinal symptoms: (1) Vomiting; (2) vomiting of worms; (3) passing of worms by mouth and bowel; (4) diarrhea, oftentimes blood-streaked; (5) anorexia; (6) passing of worms by bowel; (7) abdominal pain; (8) abdominal distension; (9) prolapse of rectum; (10) tenesmus; (11) constipation.

D. Pulmonary symptoms: (1) Cough; and (2) dyspnea.

Thus, the symptoms of infection with 2 or more intestinal parasites were the accumulation of symptoms of the individual infections (table 6).

Physical findings were less important than symptoms in the diagnosis of helminthiasis. Several important signs were observed, however. Underweight was common in ascariasis and polyparasitism particularly in the younger age group. It was observed in ancylostomiasis and trichuriasis when infection was heavy but not in oxyuriasis. Abdominal distention occurred in severe ancylostomiasis, ascariasis, and when these infections were combined in the same individual. Pallor and anemia occurred in ancylostomiasis, but infantilism was not observed. Prolapse of the rectum was described in connection with the symptomatology of trichuriasis. The dehydration and loss of weight in the diarrhea of strongyloidiasis and trichuriasis were commensurate with the severity and duration of the diarrhea. Local evidences of scratching of the perianal region were observed in oxyuriasis.

#### LABORATORY FINDINGS

*Hemoglobin*—Estimations were made by the Tallqvist technic, and checked by the Sahli method when found below 50 per cent. The values obtained were contrasted with the percentages of hemoglobin in 30 children admitted to the surgical service with simple, uncomplicated fractures and free from medical disease. They compared favorably with values in the small comparative group, although hemoglobins below 60 per cent were more common in parasitized than in nonparasitized individuals. The average values are shown in tables 7 and 8. Anemia occurred most frequently in ancylostomiasis and trichuriasis, but it was not an important feature of ascariasis, oxyuriasis and strongyloidiasis. The severest anemias were noted in ancylostomiasis. Two patients with uncomplicated ancylostomiasis had hemoglobins of 10 per cent. One of these was reported in another paper (4), but the other was in the hospital recently and was not included in this study.

*Erythrocyte counts*—These corresponded closely to hemoglobin values.

*Leukocyte counts*—Although wide variations in the total number of leukocytes were observed, the average was within normal limits (table 8). In most instances deviations from the normal count were caused by unrelated presenting diseases.

*Differential leukocyte counts*—As with total leukocyte counts, wide variations in differential counts were observed, but the average was within normal limits (table 8). Special consideration was given to the number of eosinophils, since an increase has been considered of value in the diagnosis of helminthiasis. Todd

TABLE 7

*Hemoglobin determinations in children infected with intestinal parasites*

Complete blood counts were available in only 123 of the 125 patients with ascariasis.

PER CENT HEMOGLOBIN	PER CENT OF PATIENTS						
	Ancylostomiasis	Ascariasis	Oxyuriasis	Strongyloidiasis	Trichuriasis	Polyparasitism	Comparative group
10	1.4	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	1.2	0.0
30	0.0	0.8	0.0	0.0	0.0	0.6	0.0
40	4.5	4.8	0.0	9.0	4.0	3.7	0.0
50	8.5	10.4	1.5	27.0	12.0	14.9	6.0
60	51.8	56.0	35.5	27.0	36.0	39.1	35.0
70	32.4	26.4	61.5	37.0	48.0	40.4	59.0
80	1.4	0.0	1.5	0.0	0.0	0.0	0.0

TABLE 8

*Average values of hemoglobin, erythrocyte, leukocyte and differential counts in children infected with intestinal parasites*

PARASITIC INFECTION	ANCYLOSTOMIASIS	ASCARIASIS	OXYURIASIS	STRONGYLOIDIASIS	TRICHURIASIS	POLYPARASITISM
Hemoglobin (per cent).....	61	65	68	59	67	62
Erythrocytes (million).....	3.5	3.8	3.9	3.5	3.6	3.6
Leukocytes (thousand).....	7.5	9.2	6.8	8.4	8.1	9.5
Neutrophils (per cent).....	64	63	59	61	67	62
Lymphocytes (per cent).....	33	35	39	36	30	35
Eosinophils (per cent).....	2.6	1.7	3.0	2.9	2.7	3.2

and Sanford (8) stated that the normal number of eosinophils varies from 1 to 4 per cent. The percentages of patients in this study with eosinophilia of over 4 per cent were as follows:

PARASITIC INFECTION	PER CENT OF PATIENTS
Ancylostomiasis.....	1.4
Ascariasis.....	11.2
Oxyuriasis.....	18.5
Strongyloidiasis.....	18.2
Trichuriasis.....	24.0
Polyparasitism.....	18.6

*Examination of stools*—Ancylostomiasis, ascariasis and trichuriasis were diagnosed by demonstrating the characteristic ova in the stool. Strongyloidiasis was diagnosed by demonstrating the rhabditiform larvae of *S. stercoralis* in the stool. Oxyuriasis could not be diagnosed satisfactorily by examination of the stool. Although stools of 120 of the 200 patients with oxyuriasis were examined, ova were demonstrated only once. Thirty-six per cent were diagnosed by observing *E. vermicularis* on the perianal region; the remaining 63.5 per cent were diagnosed by the cellophane tip method of Brady and Wright (9). More recently the scotch tape technic of Von Hope (10) has been tried and found to be at least as satisfactory as the cellophane tip.

#### TREATMENT

In evaluating the efficacy of therapy, it was felt that failure to demonstrate ova in the stools or on cellophane tips did not prove conclusively the absence of intestinal parasites. After completion of treatment in the hospital, most of these children returned to their homes. Although examination of stools revealed no ova at time of discharge, it was not possible to determine whether an existing infection was a reinfection or an old infection incompletely treated. The following vermifuges were used during the period covered by this survey: (1) Oil of chenopodium; (2) tetrachloroethylene; (3) hexylresorcinol; (4) gentian violet medicinal; (5) infusion of quassia chips; and (6) leche de higueron. The technics found most useful and results of treatment are presented in detail.

1. *Oil of chenopodium*—This was given in doses of 2 to 3 minims per year of age. Treatment was carried out in the following manner: Magnesium citrate was administered at 11 a.m. on the day before treatment was to begin. Breakfast was withheld on the following day, the drug was given at 6 a.m. and was followed in two hours by a second dose of purgative. Food was withheld until brisk catharsis had occurred. Vomiting, intestinal cramps and prostration occurred frequently. Oil of chenopodium was used for 6 patients with ancylostomiasis, 8 with ascariasis and 9 with polyparasitism. Infection persisted in 10 necessitating changes to other more effective and less toxic drugs. Within the past year the use of oil of chenopodium has been discontinued.

2. *Tetrachloroethylene*—This was given in doses of 3 minims per year of age. Treatment was carried out in the following manner: Magnesium citrate was given the night before treatment was to begin; breakfast was withheld on the following morning, and the measured amount of vermifuge was administered under a layer (a full dose) of magnesium citrate. Untoward symptoms consisting of abdominal cramps, anorexia, diarrhea, prostration and vomiting were noted infrequently. Tetrachloroethylene was used for all of the parasites studied with the following results:

A. Oxyuriasis; the drug was used for 19 or 9.5 per cent of the patients. All of the children who returned for observation after treatment had symptoms, worms visible on the perianal region or positive cellophane tips. Tetrachloroethylene was of no value for oxyuriasis.

B. Ascariasis; the drug was used for 15 children with unsatisfactory results,

and after the drug had been given to this small group of patients its use as an ascaracide was discontinued.

C. Ancylostomiasis; the drug was used for 32 or 45 per cent of the patients, and found to be highly effective as an ancylostomacide.

D. Trichuriasis and strongyloidiasis; the drug was used in 1 patient with trichuriasis and in 1 patient with strongyloidiasis with unsatisfactory results.

E. Polyparasitism; hexylresorcinol followed in several days by tetrachloroethylene was most effective for treatment of children who had both ascariasis and ancylostomiasis. The initial use of tetrachloroethylene under such circumstances caused the ascarides to become overactive and resulted in abdominal pain. In order to eliminate this, hexylresorcinol was administered first and followed in several days with tetrachloroethylene when necessary.

S. *Hexylresorcinol*—This drug was available in 0.1 and 0.2 Gm. hard gelatin capsules. The doses were 0.1 Gm. per year of apparent age, with a maximum dose of 1 Gm. Treatment was carried out in the following manner: A preliminary laxative of magnesium citrate was administered at noon on the day preceding the beginning of treatment. A light evening meal was given. On the following morning breakfast was withheld, and at 6 a.m. the correct number of capsules was given as one dose. A laxative, magnesium citrate, was given twenty-four hours after treatment to remove moribund and dead worms. If severe infection was present, an enema was given in addition to the second dose of citrate. The capsules were either swallowed whole with water, or with younger patients, inserted directly into the esophagus by a physician. This procedure prevented rupture of the capsule, with consequent chemical irritation of the lips and mouth. Aside from infrequent vomiting, no serious toxic symptoms were observed. One patient vomited shortly after the drug was administered, and severe irritation of the lips and mouth occurred. Hexylresorcinol was used for all of the parasites studied with the following results:

A. Oxyuriasis; the drug was used in one or more courses for several children. Hexylresorcinol was found to be ineffective, and after the drug had been given to this small group of patients its use as an oxyuricide was discontinued.

B. Ascariasis; the drug was used for 98 or 78.4 per cent of the patients. Although follow-up care for this group was inadequate, no treated patient was discharged from the hospital until examinations of the stools revealed absence of ova. To 11 patients, two courses, and to 2 patients three courses of hexylresorcinol were administered before the stools became negative for ova. Of the various vermifuges used in the treatment of ascariasis, hexylresorcinol seemed to be the safest, most efficient, and certainly the easiest to administer.

C. Ancylostomiasis; the drug was used in one or more courses for 59 or 83 per cent of the patients. The stools became negative for ova in 27 or 38 per cent. It was concluded that hexylresorcinol was a good ancylostomacide although less effective than tetrachloroethylene.

D. Trichuriasis and strongyloidiasis; the drug was used for 34 or 68 per cent of the patients with the former infection and for 6 or 54.5 per cent of those with the latter infection. In none was there complete elimination of parasites. There



may have been a reduction in number of parasites since symptoms were improved.

E. Polyparasitism; the drug was used in one to five courses for 122 or 75.2 per cent of the patients. It was most effective in the treatment of ascariasis but somewhat less effective in ancylostomiasis. The effect on strongyloidiasis and trichuriasis may have been in the nature of a reduction in number of parasites. Occasionally, however, larvae of *S. stercoralis* disappeared from stools after intensive therapy with hexylresorcinol and tetrachloroethylene.

4. *Gentian violet medicinal*—This drug was used in the form of enteric coated tablets in doses of from 0.25 (0.015 Gm.) to 1 grain (0.06 Gm.) three times a day twenty minutes before each meal for two periods of eight days each, interrupted by a rest period of seven days. This treatment was used only in children old enough to swallow the capsules intact. Anorexia, abdominal cramps, nausea and vomiting occurred frequently when the larger dose of 1 grain (0.06 Gm.) was given. Gentian violet medicinal was used for all of the parasites studied with the following results:

A. Oxyuriasis; the drug was used for 76 or 38 per cent of the children. Fourteen of these were followed carefully and treatment was considered as having been successful in 13. The use of the drug was discontinued in 5 additional patients because of vomiting. The criteria for determining success of treatment were: (1) absence of visible worms on the perianal region, (2) cessation of symptoms, and (3) repeated negative cellophane tips. Treatment with gentian violet medicinal was supplemented by applying a 5 per cent ointment of ammoniated mercury to the ends of the fingers and perianal region twice daily. The cause and methods of auto-infection were explained to parents, and special instructions concerning the necessity for keeping fingernails clipped short were given.

B. Ancylostomiasis, ascariasis, and trichuriasis; the use of gentian violet medicinal in these infections was without benefit.

C. Strongyloidiasis; the drug was used for 2 patients. Although severe symptoms were improved, this effect was apparently due to reduction in number rather than to complete elimination of parasites.

5. *Infusion of quassia chips*—This was prepared by boiling 3 tablespoonfuls of quassia chips in 1 quart of water until 1 pint was left. The infusion was strained and used in amounts of 2 to 3 ounces (60 to 90 cc.) as a rectal injection followed in twenty minutes by a soapsuds enema. Injections were repeated on alternate days for two months. This routine was used for 41 or 20.5 per cent of the patients infected with *E. vermicularis*. It proved to be of moderate value in those unable to swallow the tablets of gentian violet medicinal. Treatment with infusion of quassia chips often relieved symptoms but less frequently eliminated the parasites.

6. *Leche de Higuera*—Two varieties were used; (1) "Higueronia" a Colombian proprietary preparation preserved with sodium benzoate, and (2) the fresh latex obtained from the wild fig tree (*Ficus laurifolia*) which grows locally. The fresh preparation was kept at 40°F. without the addition of preservative. Treatment was carried out in the following manner: Magnesium citrate was given the night before treatment was to begin; breakfast was withheld on the following morning

and 1 to 3 ounces (30 to 90 cc.) of either the proprietary or fresh preparation were given. A laxative, magnesium citrate, was given twenty-four hours after treatment. This routine was repeated at intervals of five days. Both preparations were tried repeatedly, and both were equally ineffective in the treatment of oxyuriasis and trichuriasis. However, the success which Caldwell and Caldwell (11) as well as Faust (12) have had in the treatment of trichuriasis with this drug has encouraged further trial.

Recently 2 infants were admitted to the hospital because of persistent diarrheas associated with massive infections with *T. trichiura*. Both were treated unsuccessfully with repeated courses of gentian violet medicinal, hexylresorcinol, retention enemas of 1:1,000 solution of hexylresorcinol, and eight courses of fresh leche de higueron. Following these treatments, proctoscopic examinations were done. It was observed that the wall of the lower portion of the descending colon and sigmoid in both infants was covered with an almost continuous moving film of trichurides so that the mucosa was visible only in small spots. Then retention enemas of 3 ounces (90 cc.) of fresh Leche de higueron were given carefully on alternate days. Since Ross (13), and Getz (14), demonstrated that the necks of trichurides were sewn through small segments of the intestinal mucosa like basting threads, this procedure seemed doomed to failure. However, from 50 to 200 parasites were expelled with each enema. The first child received five enemas and was discharged improved although ova persisted in her stools. The second child received six enemas. Her weight which had dropped to 11 pounds in the hospital increased to 16 pounds when she was discharged eight months after admission. Injections with solutions of soap, normal saline and sodium bicarbonate tried as controls yielded from none to not more than 6 or 8 parasites.

Treatment of intestinal parasitism consisted of 2 important parts: first, that directed at the parasitic infection; and second, that directed at the general condition of the child. Anthelmintic therapy has been described in detail. Balanced diets adequate in calories and vitamins were given as soon as tolerance of the gastrointestinal tract permitted. Iron was administered when mild anemia was present. Blood transfusions were life saving measures in several instances of severe anemia in ancylostomiasis. Frequently it was surprising to see how soon after the beginning of treatment the children began to look brighter, became more energetic and acquired ravenous appetites.

#### COMMENT

*Symptoms*—The clinical features of each infection can be explained by keeping in mind the point of attack of each parasite. For example, *E. vermicularis* produces symptoms by wandering outside the anus and irritating the surrounding skin. *S. stercoralis* and *T. trichiura* produce irritation of the bowel. *A. lumbricoides* robs the infected individual of nutrition. In its larval form it invades various organs of the body, and the symptoms present depend upon the organs invaded. Adult ascarides in the gastrointestinal tract act like movable foreign bodies producing mechanical difficulties, or by their perigrinations perforate or invade organs associated with the gastrointestinal tract. The crux of the symp-

tomatology of ancylostomiasis lies in the fact that the parasite ingests blood, depriving its host of necessary iron.

Familiarity with the point of attack of each of these parasites made it possible in many instances to make a diagnosis of the parasite present in a child before his stools were examined. In polyparasitism, the clinical picture was not so clear. Although the symptoms and signs could not be analyzed in a simple manner, a resemblance to those produced by infection with a single parasite often made possible identification of the dominant parasite.

Ascariasis was often associated with underweight or malnutrition. Underweight was observed occasionally in severe ancylostomiasis but was more frequent when ancylostomiasis and ascariasis occurred in the same individual. Among the 161 children with polyparasitism were 68 who had ancylostomiasis and ascariasis simultaneously. Of these 28 or 41.2 per cent were underweight. In massive infections with *T. trichiura*, when persistent diarrhea was present, underweight was also observed.

A careful study of the frequency of convulsions was made. This symptom was not observed in ancylostomiasis, strongyloidiasis or trichuriasis. Convulsions occurred in 6 children with ascariasis, 2 with oxyuriasis and 3 with polyparasitism, a total of 11 or 1.8 per cent of 618 infected with intestinal parasites. These 11 patients were admitted to the hospital with temperatures ranging from 102 to 106.4°F. Four had acute suppurative otitis media, 3 had estivoautumnal malaria, and the remaining 4 had no apparent disease other than ascariasis. Thus, 7 patients had acute infections which offered more tenable explanations for this symptom than did intestinal parasitism.

In this study evidence of invasion of the appendix by intestinal parasites was meagre. In 1 child, several ascarides entered the peritoneal cavity through a perforation in the appendix and resulted in a fatal generalized peritonitis.

To clarify the rôle of helminthiasis in the etiology of appendicitis, it was considered advisable to review all of the appendectomies performed on children during the period covered by this report. A total of 35 consecutive appendixes removed from children were studied: Seven contained *E. vermicularis*; none contained *A. lumbricoides*.

Faust (15) stated that the "worm burden in the average individual infected with *T. trichiuris* is usually low (4 to 12 worms) and the clinical effects comparably slight." Getz (14) pointed out that although the number of *T. trichiura* harbored by older children or adults may be small, young children on the Isthmus of Panama were more heavily infected. Getz (14) reported 4 children in whom 1,100, 1,700, 4,100, and 400 parasites were found at autopsy. Usually trichuriasis is a mild disease, but when infection is heavy it may become a serious condition. Anemia was not an important feature in 50 children with trichuriasis (3). However, all of Getz's patients had anemia and 3 of them had prolonged diarrhea. Although he stated that prolonged diarrhea and anemia may eventuate in cardiac failure and death, he failed to explain the mechanism by which anemia developed in massive infection with *T. trichiura*.

Examination of the blood in this series of 618 patients demonstrated that there

were no characteristic hematologic findings in helminthiasis. Anemia was common in ancylostomiasis, and occasionally in severe strongyloidiasis, trichuriasis or polyparasitism. Leukocyte counts were unaffected. The value of eosinophilia in the diagnosis of helminthiasis has been stressed repeatedly by many observers. In this study, 69 or 11.2 per cent had an eosinophilia of over 4 per cent. The frequency with which this condition was observed did not warrant attaching unusual significance to it. When an eosinophilia is noted, a search should be made for intestinal parasites; the absence of eosinophilia does not imply the absence of intestinal parasites.

*Treatment*—Hexylresorcinol was most effective in the treatment of ascariasis, but less effective in ancylostomiasis. In oxyuriasis, strongyloidiasis and trichuriasis, this drug may have reduced the number of parasites. When combined with the use of a 1:1,000 solution of hexylresorcinol as a retention enema the effect was enhanced. Tetrachloroethylene was highly effective in the treatment of the other helminthic infections. When ancylostomiasis and ascariasis occurred simultaneously in the same child, hexylresorcinol was used first, and followed in several days with tetrachloroethylene when necessary. Gentian violet medicinal was of definite value in the treatment of oxyuriasis and strongyloidiasis, but it did not always eradicate these infections. Large doses of gentian violet medicinal could not be given frequently because of vomiting. Enemas of infusion of quassia chips often relieved the symptoms of oxyuriasis but infrequently eliminated the parasites. The use of Leche de higueron orally in trichuriasis was disappointing. More recently, preliminary trials of this drug as retention enemas have yielded encouraging results.

In the treatment of ancylostomiasis, ascariasis and strongyloidiasis only those parasites in the lumen of the gastrointestinal tract can be eliminated. Larvae en route to this area are not affected. Stools of patients with migratory larvae must be examined at monthly intervals for three months, and treatment administered again if ova or larvae are found. Many of the patients returned to their original environments where the likelihood of reinfection was great. However, treatment greatly decreased their burdens of worms and gave them a fresh start which carried them along until reinfection occurred. As these children become older, they do not seem to become infected so severely and are able to withstand infection better than younger children.

Before treatment can be considered fully successful, at least 2 things must be accomplished; first, the burden of worms must be eliminated or appreciably reduced, and second, the individual must be protected against reinfection. The first of these has been considered. To accomplish the second, provision must be made for the sanitary disposal of human feces. Only by that means can the vicious cycle of infection, pollution of soil and reinfection be broken. Prevention of ancylostomiasis, ascariasis, strongyloidiasis and trichuriasis is an integral part of rural sanitation, with proper disposal of feces by means of flush toilets or properly constructed latrines. Therapy must be directed not only to the patient, but to the source of his infection as well. There can be no adequate therapy as long as foci of infection remain a constant menace.

## CONCLUSIONS

The common helminthic infections observed in children living on the Isthmus of Panama are ascariasis, ancylostomiasis, trichuriasis, oxyuriasis and strongyloidiasis.

Patients are seen by physicians primarily for diagnosis and treatment of associated unrelated diseases and not because of intestinal parasitism. Helminthiasis either causes no serious symptoms, or is productive of symptoms which parents ignore or dismiss.

The clinical features of each infection can be explained by bearing in mind the point of attack of each parasite. Familiarity with these features enables the physician in some instances to make a diagnosis of what parasite is present before the stools are examined. The symptoms and signs observed in children infected with more than 1 intestinal parasite often bear a strong resemblance to those observed in the child infected with a single parasite thus facilitating identification of the dominant parasite.

There are no hematologic findings characteristic of helminthiasis, although eosinophilia occurs in over 11 per cent of the patients.

Hexylresorcinol is the most effective vermifuge in the treatment of ascariasis. Tetrachloroethylene is most effective in ancylostomiasis. None of the drugs used seemed completely effective in oxyuriasis, strongyloidiasis, or trichuriasis.

Helminthiasis is intimately related to contact with a soil polluted with embryonated ova. The incidence of infection cannot be materially reduced unless contamination of the soil is eliminated. Sanitary disposal of feces is of vital importance in any plan directed at prevention of infection of children with intestinal parasites.

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# AN EFFICIENT CONCENTRATION METHOD (AEX) FOR DETECTING HELMINTHIC OVA IN FECES (MODIFICATION OF THE TELEMANN TECHNIC)

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During the course of a survey for intestinal helminths, it was noted that 95 per cent of the infections with *Ascaris lumbricoides* which showed only infertile (virgin) eggs by dilution counting, were being missed by Lane DCF. Comparative tests demonstrated that in this respect Willis and zinc sulphate floatation as well as the Telemann (with 40 per cent) hydrochloric acid-ether and the de Rivas acetic acid-ether concentration methods were similarly unreliable.

As a result of studies thus initiated a modified Telemann technic (called AEX) was gradually devised which consistently demonstrated infertile *Ascaris* eggs. It possessed a similar degree of effectiveness for hookworm, fertile *Ascaris*, *Trichuris*, *Hymenolepis nana* and *Schistosoma japonicum* ova.

Our experience so far with AEX (acid-ether-xylol) suggests that by means of it one can accomplish the diagnosis of all types of helminthic infections which show eggs in the feces. This rests on the fact that AEX does not on the one hand, inherently preclude the demonstration of eggs which cannot float in solutions of high specific gravity, and does not on the other cause such loss of eggs from the material to be examined as occurs in the Telemann procedure described by Mathieson and A. M. Stoll (1945) and by Weller and Dammin (1945).

Our modification of the acid-ether centrifugation method is designed to accomplish certain definite objectives: the original portion of stool specimen employed is relatively large and tends to overcome the effect of unequal distribution in feces of such eggs as *Schistosoma*<sup>3</sup>; the initial portion used is measured and comminuted in a known amount of water or physiological salt solution; freeing of the eggs from the fecal matrix is fostered by allowing the suspension to stand several hours or overnight; the amount of fecal mixture taken for diagnosis is small, thus producing a small sediment derived from a known amount of the original fecal specimen; the concentration of hydrochloric acid is one that has a

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<sup>3</sup>If the presence of *Schistosoma* eggs is suspected, choose fecal material from the outer surface of the stool. When refrigeration is lacking to store the dilution flasks, hatching of miracidia is prevented by using 0.85 per cent solution of sodium chloride instead of water for the displacement and comminution phases.



specific gravity lower than that of eggs found in feces; the addition of xylol to the ether reduces the adhesive properties of the fecal coagulum, thus facilitating the passage of the eggs through it and likewise prevents foaming on shaking, thereby avoiding accidental loss of material from the tube; and the final sediment is sufficiently small in amount so that all of it can be examined, thus giving a quantitative status to the usual qualitative microscopic findings.

#### AEX METHOD

The steps in AEX are as follows:

1. Measure 4 ml. (or grams) of feces into a dilution counting flask which has been filled to the 56 ml. mark with water.

2. Add several glass beads (6 mm.), and after giving the preparation an initial shaking, set aside for several hours or overnight (preferably refrigerate); complete the comminution of the feces by vigorous shaking, so that all the eggs are free.

3. Shake to produce a thorough distribution of the eggs in the fecal suspension and transfer immediately 1.5 ml. of the suspension to a 15 ml. pointed centrifuge tube.

4. Add 3.5 ml. of 20 per cent hydrochloric acid,<sup>4</sup> put a rubber stopper in the tube and shake for one minute; allow to stand for two minutes.

5. Add 5 ml. of a freshly prepared mixture of equal parts ether and xylol and again shake for one minute.

6. Centrifuge at 1800–2000 RPM for two minutes and allow the centrifuge to come to a stop gradually without interference.

7. Separate the semi-floating coagulum from the walls of the tube with a thin wooden applicator.

8. Decant quickly, permitting the sediment in bottom of tube to remain undisturbed. Then, while holding the tube almost horizontally, clean any adhering coagulum from the inside of the tube with an applicator covered with gauze.

9. Add one drop of 0.1 N solution of sodium hydroxide to the sediment, mix thoroughly with a capillary pipette, transfer entire suspension to a glass slide and place a coverglass on it. If the density or amount of residue warrant it, make two drops of the material and cover each with a coverslip in order to secure a preparation which will allow the eggs to be seen easily. We prefer a 1½ x 3 inch slide and a 22 x 30 mm. or 25 mm. square coverslip.

10. Examine the whole preparation for eggs. Those found are from 0.1 gram of the original fecal specimen.

*Addendum for Egg Counting.* At will, or whenever eggs are demonstrated, the displacement flask preparation may be prepared for routine dilution egg counting. In order to secure an approximate decinormal sodium hydroxide suspension without appreciably altering the original dilution, one hundredth the volume of ten times normal sodium hydroxide<sup>5</sup> is added. Thus, if 1.5 ml. has been removed

<sup>4</sup>20 ml. of concentrated hydrochloric acid diluted to 100 ml. with distilled water. The net concentration of HCl in the centrifuge tube is reduced to 14 per cent.

<sup>5</sup>Prepared by dissolving 40 grams of sodium hydroxide sticks in distilled water q.s. to 100 ml. Note that this concentration is caustic.

from the 60 ml. suspension, 0.58 ml. of the ten times normal sodium hydroxide is added. One can proceed at once with dilution counting, although we usually allow the flask with its contents to stand for an hour after shaking.

Workers familiar with dilution egg counting will recognize that steps 1 and 2 are based on the use of the Stoll and Hausheer (1926) displacement flask as a means of starting with a relatively large portion of feces, measuring it neatly and then providing ample opportunity for the eggs of the parasites to be released from the fecal constituents. Step 3 samples the resulting egg population in the fecal suspension, the 1.5 ml. employed representing 100 mgm. of the fecal specimen. This use of 0.1 gm. of feces in AEX means that eggs present at a rate of 10 or more per gram should be demonstrable. We know of no procedure which is so consistent in routine practice in producing positive results from fecal specimens containing so few eggs.

Step 4 deserves special consideration. Telemann<sup>6</sup> originally recommended concentrated hydrochloric acid in order to accelerate the disintegration of feces. It is known to users of the Telemann technic that the tube, after being shaken with acid and ether and then being centrifuged, shows four layers: (1) an ether supernate resting on (2) a semi-floating plug of coagulum, which in turn rests on the surface of (3) the acid; at the bottom of the acid is the (4) sediment which is used for examination. In an analysis of low egg yields from the Telemann technic used with 40 per cent HCl as compared to dilution egg counts made from aliquot portions of the same fecal specimens, we found no eggs in the ether supernate; infertile *Ascaris* eggs were found in the coagulum, however, and both hookworm and fertile *Ascaris* eggs were abundant at the interface of the coagulum and acid and in the upper layers of the acid. The presence of eggs in the upper part of the acid portion seemed to have only one meaning, namely, that the concentration of acid being employed had a higher specific gravity than that of the eggs. Accordingly, tests were made to determine what concentration of acid best contributed to the functioning of the technic but still had a specific gravity lower than 1.050, which is less than the density of the parasitic eggs commonly found in feces. Hydrochloric acid of specific gravity 1.030 was

<sup>6</sup>During the period we were perfecting AEX we did not have access to the original report of Telemann (1908), which recommends placing pea-sized portions of feces from 5 parts of the stool in a reagent glass filled with a 1:1 "Gemisch" of ether and pure hydrochloric acid. After shaking, this is filtered through a fine sieve, centrifuged for about one minute and the sediment examined. Telemann adds in a footnote that in many cases the clarity of the preparation can be increased by re-shaking the sediment with the ether-HCl mixture or with water and again centrifuging. Weller and Dammin (1945) used the Telemann in "routine work" with "a pea-sized fragment of feces emulsified in 5 cc. of 40 per cent HCl (40 cc. conc. HCl diluted to 100 cc.); . . . the material was filtered through two layers of moist gauze . . . and an equal quantity of ether was added," etc. In semi-quantitative comparisons with a "basic suspension" (feces diluted with an equal volume of physiological saline), they used one cc. of basic suspension with 5 cc. of 40 per cent HCl. Mathieson and A. M. Stoll (1945) shook 1 gm. samples of feces in "5.0 ml. of approximately 15 per cent HCl (40 ml. of conc. HCl made up to 100 ml. with distilled water)." The calculated specific gravities of the acid-feces mixtures used are: Telemann's original, about 1.180 (equivalent to the conc. HCl which is immiscible with ether); Weller and Dammin's "routine" modification, 1.070; their semi-quantitative modification, 1.060; Mathieson and A. M. Stoll, 1.070.

found best adapted to this result. It is produced by the addition of 3.5 ml. of 20 per cent hydrochloric acid to the 1.5 ml. of aqueous fecal suspension referred to in step 3. When this lower concentration of acid was used it proved to have an incidental effect of considerable practical convenience. It had been noticed that, in mounting the sediment for microscopic examination from the Telemann 40 per cent HCl technic, there were frequently present crystals which tended to cause the coverglass to lie in a plane not parallel to the slide. The nature of these crystals was undetermined, but they were not encountered with the concentration of acid employed in AEX.

In step 5 by adding and shaking equal parts of xylol and ether instead of ether alone, we not only reduced the messy character of the Telemann procedure at this point but diminished the adhesive character of the coagulum. Foaming, common when ether is used alone, and consequent loss of material from the tube were avoided. This happened to have been the first modification of the Telemann which we undertook.

The centrifugation intensifies the characteristic layering and leaves the small, egg-containing sediment in the bottom of the tube. While we prefer centrifugation at 1800–2000 RPM for step 6, the result should be attainable on a steady hand centrifuge. Steps 5 and 6 cause the gross debris to come up into the coagulum. This is one of the highly useful attributes of the Telemann technic, the net effect of which is a screening of the material. With the small amount of feces used in AEX, this screening effect of the acid-ether-xylol alone is adequate, and protects against inevitable loss of ova through added mechanical screening. With larger amounts of feces additional screening has been the rule,—a sieve by Telemann (1908), gauze by Mathieson and A. M. Stoll (1945) and by Weller and Dammin (1945). Both gauze and successive centrifugations in water are used in the modification by Faust *et al.* (1939) of the technic of Lane (1923–24).

Steps 7 and 8 get rid of the material of no interest to the microscopist. The remaining sediment may be taken up unchanged or, as we prefer in step 9, after a drop of decinormal sodium hydroxide has been added to neutralize the effect of acid fumes on the metal of the microscope.

#### PROCEDURE AND MATERIALS USED IN COMPARATIVE STUDIES OF THE EFFICACY OF AEX

Our procedure in the Parasitology Laboratory of NAMRU No. 2 was routinely to tube specimens in water in Stoll-Hausheer displacement flasks (steps 1 and 2 above). Before adoption of AEX diagnostically, DCF was regularly performed by utilizing 10 ml. of the resulting fecal suspension, equivalent to  $\frac{2}{3}$  gram of the original feces. We used a Lane four-bucket hand centrifuge (Turner) and saturated sodium chloride solution for the floatation fluid. (A test series run with zinc sulphate at sp. gr. 1.180 did not show advantages over table salt in routine DCF.)

After examination of a Lane cover, the original fecal suspension left in the displacement flask was made at will into an approximate decinormal sodium hydroxide suspension for dilution egg counting. This was done as noted in "Ad-

dendum for Egg Counting" above. The majority of dilution counts were by a single small drop, 0.075 ml., representing 5 mgm. of original feces, giving  $\frac{1}{200}$  the count per gram.

During the period when AEX was under test, an added 1.5 ml. was withdrawn from the fecal suspension before adding the sodium hydroxide. Thus AEX, DCF and dilution counts were from the same egg suspension.

When Telemann examinations with 40 per cent HCl were made, a portion of the original stool approximately one gram in amount, was placed in a test tube and 5 ml. of the acid (with specific gravity about 1.070) added. This was shaken until thoroughly disintegrated and then filtered through two layers of moist gauze into a 15 ml. pointed centrifuge tube. To this was added 5 ml. of ether and the tube again shaken. The completion of the technic followed steps 6-10 inclusive above. The amount of sediment usually required two or more coverslip preparations for complete examination. We are indebted to W. D. Lindquist, CPhM, for most of these Telemann controls.

In order to insure objectivity on the tests, the technics were handled independently in routine laboratory fashion and comparisons made afterward.

Results on two groups of fecal specimens are presented, which illustrate the efficacy of AEX.

1. The first is from a series of 100 consecutively received fecal specimens that came to the Parasitology Laboratory of NAMRU No. 2 for routine examination, together with a series of 34 specimens sent in specifically for diagnosis of *Schistosoma japonicum*. The latter were of special interest to us, having been sent to our laboratory from an army hospital where the Telemann technic with 40 per cent HCl had revealed only one positive. They were from twenty patients who had been diagnosed in the field as having schistosomiasis some two to six months previously; fourteen of them had received treatment with fuadin from one to five months previously.

2. Two hundred fifty consecutively received fecal specimens from natives of Okinawa were examined for helminthic ova in the field by means of the AEX method and dilution egg counting. A. J. Barger, PhM1/c, and E. Fong, PhM1/c who performed these examinations under the supervision of Lt. Comdr. M. B. Franks, MC, USNR, were well trained in parasitological examinations, but had had only a few days practice with AEX before departing for Okinawa.

## RESULTS

Detailed analysis of the efficacy of AEX, Telemann with 40 per cent HCl and Lane DCF with one cover, compared to dilution egg count results on specimens from Group 1, is given in table 1. AEX proved superior to the others in establishing a diagnosis as well as in egg recoveries.

In 18 specimens negative to hookworm on one dilution slide AEX revealed 89 per cent of the infections (sum of positives of three concentration methods) as compared to 50 and 39 per cent for the Telemann and DCF methods, respectively; in equally light egg-bearing feces AEX showed *Trichuris* present in 15 specimens, while the Telemann and DCF revealed 53 and 73 per cent.

TABLE 1  
Comparative efficacy of AEX, Telemann and Lane DCF (one cover) techniques in demonstrating ova of helminths in feces

	NUMBER OF SPECIMENS*	AVERAGE (AND RANGE) OF DILUTION EPG†	AVERAGE (AND RANGE) OF EGG RECOVERY EXPRESSED AS EPG‡			DIAGNOSTIC EFFICIENCY: PER CENT OF SPECIMENS POSITIVE BY		
			AEX	Telemann	DCF	AEX	Telemann	DCF
Hookworm	18	Neg.	48[2 Neg.] (10-170)	8[5 Neg.] (2-21)	27[11 Neg.] (3-75)	89	50	39
Hookworm	7	200	84 (20-160)		18[3 Neg.] (5-100)	100		57
Hookworm	23	640 (250-1000)	313 (40-700)	135 (30-400)	218[1 Neg.] (5-520)	100	100	96
Hookworm	10	5000 (1100-19500)	2000 (410-10800)	500 (50-3900)	1200 (100-2900)	100	100	100
<i>Ascaris</i> , fertile	6	5600 (200-9800)	1700 (350-2700)	140 (8-240)	560 (12-620)	100	100	100
<i>Ascaris</i> , infertile	13	1500 (150-4600)	500 (30-590)	0 [Neg. 2 only]	0 [13 Neg.]	100	None positive	None positive
<i>Trichuris</i>	15	Neg.	54 (20-110)	21[5 Neg.] (11-28)	9[4 Neg.] (3-26)	100	50	73
<i>Trichuris</i>	14	500 (200-1000)	165 (20-500)	38 (1-50)	24[2 Neg.] (3-120)	100	100	86
<i>S. japonicum</i>	9	Neg.	17 (10-40)	[9 Neg.]	[9 Neg.]	100	None positive	None positive
<i>S. japonicum</i>	10	400 (200-1600)	100 (20-230)	1 positive [9 Neg.]	[10 Neg.]	100	10	None positive

\* All were examined by AEX and DCF. Account is taken in the "diagnostic efficiency" column of the net number of Telemanns examined.  
† The majority of the dilution counts were on the basis of one small drop (0.075 ml.) with a factor of 200 to express EPG (eggs per gram) of original fecal specimen.

‡ The EPG as given in Table 1 were computed as follows: AEX counts (made on 1.5 ml. of a 1/2 dilution of original feces, thus representing 0.1 gram) were multiplied by 10; Telemann counts were based on the approximately one gram of original feces used in performing the technique (the amount of sediment usually required several coverslip preparations to secure complete examination); DCF counts (begun with 10 ml. of a 1/2 dilution of original feces, thus representing 1/2 gram) were multiplied by 14. Figures in brackets represent specimens with no egg recovery.

Considering all the dilution-positive specimens AEX demonstrated on the average  $\frac{1}{3}$  to  $\frac{1}{2}$  of the expected yield of nematode ova. The Telemann technic with 40 per cent HCl yielded much fewer eggs, averaging only  $\frac{1}{3}$  of the AEX recoveries for hookworm,  $\frac{1}{4}$  for *Trichuris* and  $\frac{1}{12}$  for fertile *Ascaris* (respectively  $\frac{1}{5}$ ,  $\frac{1}{12}$  and  $\frac{1}{16}$  of dilution averages). Single cover Lane DCF, while superior to the Telemann technic, was likewise much less efficient than AEX, revealing an

TABLE 2

*Routine results by AEX and dilution egg counting in a field study of 250 fecal specimens from Okinawan natives*

	NUM- BER OF SPECI- MENS	AVERAGE (AND RANGE) OF DILUTION EPG	AVERAGE (AND RANGE) OF RECOVERY BY AEX EXPRESSED AS EPG	PER CENT POSITIVE BY AEX
Hookworm	14	Neg.	280 (110-1000)	100
	22	200	270 (70-440)	82
	64	650 (400-1000)	450 (40-1000)	92
	72	4,200 (1100-63,000)	*	100
<i>Ascaris</i> , fertile	10	Neg.	400 (50-1000)	100
	5	200	296 (140-680)	100
	8	600 (400-1000)	400 (140-680)	100
	64	15,300 (1100-80,400)	*	100
<i>Ascaris</i> , infertile	4	Neg.	400 (60-1000)	100
	12	450 (200-1000)	350 (70-850)	67
	13	3,000 (1100-8200)	*	100
<i>Trichuris</i>	20	Neg.	120 (30-670)	100
	15	200	180 (20-670)	100
	13	600 (400-1200)	260 (110-570)	100

AEX gave negative results in 9 dilution-positive hookworm and 4 dilution-positive infertile *Ascaris* infections averaging, respectively, 400 and 450 eggs per gram of feces.

\* Most specimens in dilution counts of 1100 EPG and over were tabulated as "over 100 eggs" for AEX, i.e. over 1000 EPG. There were, however, in this group 39 hookworm, 8 fertile *Ascaris* and 8 infertile *Ascaris* specimens counted to completion by virtue of the fact that less than 100 eggs were encountered by AEX. In these the net AEX recovery rates (compared to average dilution counts on same specimens) were: Hookworm (average 2300) 360; fertile *Ascaris* (average 3400) 510; infertile *Ascaris* (average 2500) 690.

average of  $\frac{1}{2}$  of the AEX recoveries for hookworm,  $\frac{1}{4}$  for *Trichuris* and  $\frac{1}{3}$  for fertile *Ascaris* (respectively  $\frac{1}{5}$ ,  $\frac{1}{12}$  and  $\frac{1}{16}$  of dilution averages). DCF showed no hookworm eggs in 4 of 30 egg count positives and 2 of 14 *Trichuris*.

Both Telemann with 40 per cent HCl and DCF gave negative results in infections showing only infertile *Ascaris* eggs. AEX, on the other hand, detected all the infections showing solely infertile *Ascaris* eggs and demonstrated  $\frac{1}{3}$  of the ova present by dilution egg count.

In our limited series of *Schistosoma japonicum* infections, AEX revealed ova in nine dilution-negative specimens in which Telemann (and DCF) were likewise

negative, and AEX diagnosed all of the ten dilution-positive specimens with a demonstration of  $\frac{1}{4}$  of the *Schistosoma* ova shown present which ranged from 200 to 1600 EPG. The Telemann with 40 per cent HCl gave only one (uncounted) positive, and DCF none.

In one specimen containing 1000 *Hymenolepis nana* ova per gram of feces by dilution count AEX yielded 130 ova and DCF 8. In a formalin-preserved specimen, 50 *Metagonimus* ova per gram of feces were recovered by AEX, while both the Telemann and DCF showed none.

An analysis of the results of the examinations performed in the field is presented in table 2. There were 14 hookworm, 10 fertile *Ascaris*, 4 infertile *Ascaris* and 20 *Trichuris* infections revealed by AEX that were missed by single dilution counts. In dilution-positive specimens with egg counts of less than 1100 per gram of feces, AEX revealed 90 per cent of the hookworm, 100 per cent of the fertile *Ascaris*, 67 per cent of the infertile *Ascaris* and 100 per cent of the *Trichuris* infections with recoveries of  $\frac{1}{2}$  to  $\frac{4}{5}$  of the expected number of ova. The dilution-positive specimens with 1100 or more eggs per gram of feces were not completely counted in the AEX slides, but all were positive (table 2).

In one specimen containing 600 *Taenia* ova per gram by single dilution slide AEX demonstrated in excess of 1000.

Several specimens, not included in the report of the field examination, yielded infertile *Ascaris* ova when examined by AEX. All were previously examined by means of the zinc sulphate floatation method at a neighboring hospital with negative results.

#### DISCUSSION

Much of the emphasis in improvement of fecal examination methods in the last three decades has centered on diagnosis of the intestinal nematodes, with special reference to hookworm. Global dispersal of military personnel in World War II has brought increased exposure to other helminths as well, and expert laboratory diagnosis cannot stop now with techniques which fail to detect them routinely. It is obvious that the technical approach which depends upon floating nematode ova in fluids of high specific gravity does not apply equally to operculate cestode or trematode ova, and may not to others. This is true whether eggs are merely permitted to rise in saturated salt solution (Kofoed and Barber, 1918; Willis, 1921), or are forced to rise due to the increased gravitational field provided by centrifuging them in a strong solution of sodium chloride (Lane, 1924) or zinc sulphate (Faust *et al.*, 1939) or cupric nitrate (Garcia and Pesigan, 1940). Lane (1928) remarked that trematode eggs float "only by accident." Moreover, a single floatation procedure has not proved dependable for all the nematode eggs. We have noted in this report that infertile *Ascaris* eggs appear only occasionally in DCF preparations, and this parallels an earlier (Stoll, 1929) demonstration of poor efficiency of the Willis technic for *Ascaris* eggs.

Scott (1937) has pointed out that in diagnosis of *Schistosoma mansoni* infections the dilution counting method (Stoll, 1923) has a place, but patently it will have diagnostic value only when the trematode egg concentration in the feces is 100 or

200 eggs per gram. Weller and Dammin (1945) and Mathieson and Alice M. Stoll (1945) have recently called attention to the value for *Schistosoma* diagnosis of Telemann's<sup>7</sup> concentration technic, which continental European workers have long recognized. It seemed to the present authors that with the messy characteristics of the Telemann improved, the specific gravity of the acid lowered, and with the quantity of material to be examined on the slide decreased, this technic could serve as an all-purpose diagnostic procedure when parasitic eggs were present in the feces in smaller numbers than dilution counting routinely detected. As already indicated AEX accomplishes this, and permits later dilution counting of the original fecal mixture at option when the eggs present are numerous.

From the standpoint of diagnosis it is clear that when eggs are numerous even a simple smear technic will reveal them (Hausheer and Herrick, 1926). When eggs are few the first hazard any diagnostic technic encounters is whether they are set free from the fecal constituents so that they will later be demonstrable. Hydrochloric acid is a superior reagent in this respect, and Telemann deserves credit for introducing it for such purpose. The high specific gravity of the strong HCl he recommended had the effect, however, of causing eggs to be lost by their rising out of the sediment in the bottom of the tube, which the microscopist eventually examined. It seems to us that the lower specific gravity of the 5 per cent acetic acid which de Rivas (1928) substituted in Telemann's procedure was essentially responsible for the increased egg yields which de Rivas demonstrated, inasmuch as acetic acid is a poorer comminuting agent than HCl.

With AEX, hydrochloric acid of sufficient dilution is utilized so that its low specific gravity does not cause loss of eggs from the sediment, and yet it is strong enough to contribute to effective freeing of eggs from the fecal material derived from the displacement flask. This resulting egg enrichment of the sediment in AEX has permitted us to greatly decrease the total amount of original fecal mixture with which we deal, and thus still further decrease the amount of fecal debris to be screened from the eggs by acid-ether-xylol; this in turn decreases the actual amount of sediment to be examined by the microscopist. Our conclusion, in brief, is that an excellent opportunity to demonstrate the ova present in an aliquot of 100 mgm. of an original fecal sample of 4 grams represents a more favor-

<sup>7</sup>Weller and Dammin table the percentage recovery of *Schistosoma mansoni* eggs from 24 "basic suspensions" of feces which contained 433 to 2133 eggs per cc. by dilution count. Their acid-ether routine with basic suspension, in which the entire sediment was examined, had an average recovery of 20 per cent of the eggs; when the sediment obtained by centrifuging was dispersed in the final cc. of acid in the tube and aliquot portions examined (referred to as acid-ether semi-quantitative test), there was an average recovery of 48 per cent of the eggs; this indicated that in "routine" the sediment was too heavy for effective microscopy. ZnSO<sub>4</sub> "loop removal" averaged 5 per cent recovery, with 2 diagnoses missed; ZnSO<sub>4</sub> "multiple coverslip" averaged 23 per cent recovery from 3 supernatant coverslips per tube (of the missing 77 per cent of ova, 39 per cent were demonstrable in the sediment, 38 per cent unaccounted for). Mathieson and A. M. Stoll compared yields of *S. japonicum* ova in dog feces by five technics performed in parallel, and demonstrated a total of 567 eggs by acid-ether, 120 by sedimentation, 25 by smear, 6 by miracidial hatching, and 2 by zinc sulphate loop removal.



able technical approach than a poor or even a good opportunity to demonstrate the ova derived from working with a fecal bolus 500 or 1000 mgm. from which the eggs are less completely freed. This obtains with all species of parasite eggs found in feces.

#### SUMMARY

1. A modification of the Telemann hydrochloric acid-ether technic is presented, in which hydrochloric acid with a specific gravity of 1.030 and a mixture of equal parts of ether and xylol, are employed. We refer to this modification as AEX.

2. The use of hydrochloric acid with a specific gravity of 1.030 provides satisfactory acid disintegration of the feces and permits ova of all species to be easily sedimented by centrifugation.

3. The addition of the xylol diminishes the adhesive properties of the coagulum of the fecal debris and prevents foaming common to the use of ether alone.

4. AEX was found to be far superior to both the Telemann and Lane DCF technics in both diagnosis and in egg recovery rates in infections showing hookworm, fertile *Ascaris* and *Trichuris* ova, while in infections showing *Schistosoma* or infertile *Ascaris* ova AEX was the only procedure which consistently demonstrated them. Diagnostically, all species of parasite eggs occurring in feces should be readily found by AEX.

5. In a helminthic survey made under field conditions AEX has proved a reliable diagnostic procedure.

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# OPPORTUNITIES FOR TRAINING AND RESEARCH IN TROPICAL MEDICINE AND PUBLIC HEALTH IN MEXICO

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## INTRODUCTION

The medical specialties of Tropical Medicine and Public Health have made rapid strides in the current century. The present global conflict has greatly increased the interest in these subjects particularly in those countries in which movement of troops from temperate or semitropical countries has occurred to strictly tropical areas. However, the lack of medical personnel with experience or training in tropical medicine and tropical public health has presented a serious problem to military strategists.

Furthermore in the future world, if the terrible lesson of the present war is to be profitable and if the enormous sacrifice of these years has not been useless, there will appear a new attitude which will not admit inferiority of the inhabitants of tropical zones but will search for the means of creating better living conditions for these individuals and therefore will face the prodigious problems which tropical diseases present.

Mexico is located between 15° and 32° North latitude but owing to the mountainous nature of its central area there is great variability in climate with altitude determining this rather than latitude. The great area and rugged nature have resulted in a lack in development of transportation in certain localities so that today some areas remain more or less isolated and have slight interchange of population with adjoining communities.

With the enactment of the present Constitution in Mexico in 1917, public health became an autonomous institution, a part of the executive division of the Federal Government. In 1943 the Departamento de Salubridad Pública through fusion with the Secretaría de la Asistencia Pública, became the Secretaría de Salubridad y Asistencia. The Secretario is a member of the Presidential Cabinet. A table of organization appears on page 530.

Mexico has been steadily increasing its appropriations for the Federal Health Department so that in 1942 there was 4.35 per cent of the Federal Budget available, exclusive of state funds (Quinoñes, 1943), for strictly public health activities. The staff comprises individuals with both national and international training. A considerable proportion of the technical personnel have been trained in the United States and others in Europe. Bilingualism is characteristic.

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leishmaniasis and intestinal protozoa. The laboratory of entomology studies arthropods which are vectors of malaria, *Onchocerca*, Leishmaniasis and various ticks. The laboratory of experimental therapeutics in cooperation with the clinical section, is investigating the therapeutic action of various new drugs in tropical diseases and the blood and urine levels of these drugs. The chemistry laboratory is conducting studies on vitamin C, sulfanilamide derivatives and D.D.T. insecticide. The typhus laboratory is investigating rapid diagnosis of various strains, a therapeutic serum, cultivation of the rickettsiae and has isolated a strain of Rocky Mountain spotted fever.

A well equipped hospital occupies the floor immediately above the laboratories of the Instituto for the hospitalization of interesting cases. These are frequently transported from distant points in Mexico. This provides the opportunity for continuous detailed clinical observation of cases of diseases being studied by the Institute staff. As field stations there are available three distinct centers of investigation for use of the Instituto de Salubridad y Enfermedades Tropicales, namely Huixtla *Onchocerca* Center, Arcelia Pinta Center and Boca del Río, each of which will be discussed separately.

#### *Onchocerca Investigations*

In 1942 there was opened a 50 bed hospital for *Onchocerca* cases in Huixtla, Chiapas. This city is located on the Ferrocarriles Nacionales at a distance of 48 kilometers from Tapachula, which is provided with an excellent airport with stops on the International flight of Pan American Airways and regular service by Compañía Mexicana de Aviación. Huixtla has an altitude of less than 50 meters above sea level. It is located just on the outer fringe of the endemic zone but within 1 kilometer of the hospital on the Pacific slope of adjoining mountains, devoted to cultivation of coffee where numerous cases of onchocerciasis are found.

The Secretaría de Salubridad y Asistencia in addition to hospitalizing the more serious cases for treatment has several brigades which go into the field, chiefly to villages and coffee fincas. Each brigade includes a doctor, nurse and various aides. Excision of nodules under local anaesthesia is practiced in these ambulatory clinics. The Huixtla hospital for onchocercosis also provides a special outpatient clinic for parasitosis in general. The strictly tropical climate of the town (population 8,000) is conducive to a high incidence of intestinal parasitic infections. An excellent laboratory forms a part of the Hospital.

The hot, rainy season in Huixtla is from April to September inclusive and visits to the area are more pleasant during the balance of the year. A guest house on the hospital grounds has just been completed and will provide lodging for official visitors.

During the past two years the Pan American Sanitary Bureau, with financial assistance from the Institute of Inter-American Affairs (Office of Inter-American Affairs) has been cooperating with the Secretaría de Salubridad y Asistencia in an active research program looking toward the control of onchocercosis. The parasitological, entomological, pathological, epidemiological and therapeutic features of the disease are under investigation.

*Pinta*

Pinta has been the object of investigation for many years in Mexico where the disease is widespread and of frequent occurrence. It is estimated that there are 300,000 cases in the country. The disfiguration is sufficient to lead to active cooperation on the part of the sufferers. In 1943 there was opened in Arcelia, Gro. a center for the investigation of Pinta. This is located at a distance of 340 kilometers from Mexico City. The highway is paved to Iguala and the intervening distance from Iguala to Arcelia (140 kilometers) is an improved all weather road.

The center includes a recently constructed civil hospital of approximately 60 beds with dispensary. In addition to study, either clinical or laboratory, at Arcelia, cases are transported to the Instituto de Salubridad y Enfermedades Tropicales in Mexico for investigation. Active studies on pathology, therapy and epidemiology of the disease are in progress.

*Boca del Río Health Center and Tropical Medicine Training Station*

In September 1944 the Secretaría de Salubridad y Asistencia, through the Dirección de Cooperación Interamericana de Salubridad Pública initiated the construction of a Health Center and Tropical Medicine Training Station at Boca del Río, Ver. The building is located on the outskirts of Boca del Río, a town of 1,500 population. It fronts on the Highway from Veracruz to Córdoba at a distance of 12 kilometers from the former city and the rear of the building commands an excellent view of the Gulf of Mexico. Two railroads connect Mexico City and Veracruz, and one of them has a station in Boca del Río. The Veracruz airport (with international and local service) is approximately 6 kilometers distant from the Training Station.

The community of Boca del Río is engaged principally in two activities, fishing and agriculture. The river entering the Gulf at this point is the means of transportation for a considerable agricultural area adjoining and furnishes the only contact with the outside.

Veracruz is a modern city of approximately 75,000 population provided with an excellent port and is one of the most important trading centers in Mexico. At present there is under construction a modern hospital of 500 beds. A well organized full-time health center is adjacent. The facilities of these institutions are available to students of the Boca del Río Training Station.

The Boca del Río Health Center and Tropical Medicine Training Station consists of a two-story building having a frontage of over 36 meters and two wings extending each for 20.5 meters. Only the central portion has two stories at present. A complete health center including dental service will be provided for the service of the local community. Additional examining and treatment rooms and laboratory space are provided for the students. Two hospital wards of 5 beds each and a smaller one of 2 beds are included. Living quarters for resident director and nurse are provided. These facilities together with dining room (capacity 24), kitchen, pantry, laundry, garage, waiting room, comprise

the first floor. The second story includes dormitory space for 20 students' library-class room and recreation room. The entire second story corridor is enclosed by copper screening as well as all doors and windows in the structure. The two wings on the second story will provide additional recreational space for trainees and opportunity for subsequent expansion.

The station in general provides three distinct types of service. First a full-time health center for the community. Hospitalization of ordinary cases will not occur—they can be provided for in Veracruz. In the second place the center will act as a field research station for the Instituto de Salubridad y Enfermedades Tropicales. The personnel of the latter may spend varying periods for investigation *in residence* at Boca del Río and if desirable use the entire facilities of the hospital for their temporary studies. Finally the structure is intended as a Tropical Medicine Field Training Station. It is available for the clinical field training of doctors interested in tropical medicine from Mexico, United States or Latin America. It is assumed that such students have already received basic training in both laboratory and clinical tropical medicine before coming to Boca del Río. Here they would have the opportunity to study tropical medicine under strictly tropical conditions, to make field (epidemiological) investigations tracing the patient's disease to the point of origin.

### *Nutritional Studies*

For several years the Secretaría de Salubridad y Asistencia has recognized nutritional defects as one of the principal public health problems in Mexico. Serious investigations have been continuously in progress both in the laboratory and clinical fields. Assistance has been rendered through the cooperation of the Rockefeller Foundation and the Kellogg Foundation. In order to coordinate present activities and expand certain of them the Instituto de Nutrición was established in 1943. A new headquarters and laboratory building will be completed and ready for occupancy early in 1946.

Briefly the program includes the following: a study of the cost of diets from a group of 340 families in a poor ward of Mexico City, a clinical and laboratory examination of nutritional status of school children; blood level of vitamins; analysis of various typical Mexican foods, dietary standards for various age groups and occupations; the preparation and collection of a variety of recipes utilizing such common foods as beans and corn.

The Kellogg Foundation with the cooperation of Massachusetts Institute of Technology has been assisting the Secretaría de Salubridad y Asistencia through the training of personnel, the obtaining of equipment and initiation of analysis of local available foods. A special project has been the attempt to devise a supplementary low cost meal for school children.

The Hospital for Nutritional Diseases, with approximately 100 hospital beds, was terminated in 1945. Complete laboratory, autopsy, laundry and kitchen facilities are provided in addition to the hospital and outpatient dispensary services. This unit affords ideal conditions for clinical studies on nutritional deficiencies.



### *Malaria and Other Parasitic Infections*

The administrative responsibility for the execution of control of malaria and other parasitic infections is in the Oficina de la Campaña contra el Paludismo, la Oncocercosis y otras Parasitosis, of the Dirección de Epidemiología. Through the sale of required stamps on all mail, funds are available to the Secretaría for the exclusive use in work for the permanent elimination of anopheline breeding places. Particular attention has been given to drainage and to intermittent irrigation for the control of *Anopheles pseudopunctipennis* in the rice fields. Technical assistance in malaria control has been rendered by the Rockefeller Foundation.

Hookworm and other intestinal parasites have constituted a serious menace to the population of Mexico. Many areas still have a high incidence. Practically all Health Centers scattered throughout the Republic have special outpatient clinics devoted to the treatment of parasitosis.

### *Typhus*

Typhus, both of the old world (classical) and new world (murine) form is present in Mexico. A special Mexican Typhus Commission has been established and a broad research program is under way. This includes clinical studies, laboratory diagnostic procedures, control of lice, etc. Excellent opportunities are thus available for special investigations of this disease. A typhus laboratory is located in the General Hospital.

### *Leprosy*

Leprosy constitutes an important public health problem since approximately 8,000 cases are estimated to occur in Mexico. A comparatively new leprosarium with capacity for 400 lepers is located at a distance of 30 kilometers from Mexico City. In addition a dispensary is situated in Mexico, D. F., which affords further opportunity for investigation. The anti-leprosy program forms an important activity of the Dirección de Epidemiología.

### *Other laboratories and institutions*

The Laboratorio Central of the Secretaría provides the central diagnostic facilities for the country. The Instituto de Higiene prepares vaccines and other biologicals. The Secretaría also is in charge of all Federal Hospitals and similar institutions. To mention only a few of interest in the field of tropical medicine: Hospital del Niño (Children's Hospital), Hospital General, Hospital Morelos (venereal disease), National Leprosarium, various tuberculosis sanatoria, etc. The active utilization of all of these institutions is readily available for students of tropical medicine.

## PUBLIC HEALTH TRAINING AND ACTIVITIES IN MEXICO

### *Escuela de Salubridad e Higiene*

The School of Hygiene was established in 1924 in Mexico City for the in-service training of public health personnel, principally physicians with some

courses for nurses and sanitary inspectors. A new building was completed in 1939 which also houses the Instituto de Salubridad y Enfermedades Tropicales. A large auditorium with seating capacity of 200, three lecture rooms, five laboratories, library, lounging rooms, etc. are available.

The course for public health officers has recently been extended to include 40 weeks, divided into four quarters of 10 weeks each, of which the last quarter is entirely devoted to field training in the health centers. The course begins the latter part of February. Facilities at present are available for 40 students but this number could easily be augmented. While the School was founded for the training of Mexican personnel it offers decided advantages for latin american physicians—similarity of problems, environment, economic resources and language. Such students are welcomed on the same basis as nationals. The integration of this course with the Health Centers used as training stations will be discussed below.

Particular emphasis is given to laboratory training in bacteriology, parasitology and biochemistry. Epidemiology, biostatistics, communicable disease control, public health administration and sanitary engineering are also stressed. The faculty is composed of full time professors, investigators of the Instituto de Salubridad y Enfermedades Tropicales and other personnel of the Secretaría de Salubridad y Asistencia. A gradually increasing proportion of the faculty are on a full time basis.

At intervals shorter courses are given for health officers unable to take the longer course. Courses of three months duration are given to public health nurses who are graduates of an acceptable nursing school. Sanitary inspectors are also given courses of three months instruction.

### *Training Stations*

A number of the best Health Centers in the country are utilized as training stations for personnel of the Secretaría de Salubridad y Asistencia. One of these, Tacuba Centro de Higiene y Estación de Adiestramiento, is located immediately adjoining the building housing the School of Hygiene and the Institute of Tropical Medicine. This institution provides health service for a region of the Federal District containing approximately 28,000 population, of one of the less wealthy parts of the Capital. It has visiting nurses as well as outpatient dispensaries, dental service, etc. providing a similar care of the people that the Eastern Health District of Baltimore, Md. does. Another training center is available in Cuernavaca, Mor., a distance of 75 kilometers from the Capital. Others are scattered throughout the country. Varying numbers of physicians, nurses or sanitary inspectors are sent to these centers at any one time for training.

On a similar scale the "basic" training centers furnish a like service. Shortly, eight of these will be in operation throughout the country. Usually they are located in smaller cities and receive three physicians, three nurses and three sanitation officers for a period of two months. These trainees are considered as apprentices who "learn by doing" and if after demonstration, in their own

health center, of ability may be selected for the longer course at the School of Hygiene in Mexico.

In the health center and training station program the Secretaría has received the collaboration of the Rockefeller Foundation.

#### *Dirección de Salubridad y Asistencia en los Estados y Territorios*

The division of the Secretaría having responsibility for administration of the Health and Welfare program throughout the country has the above name. Its activities are coordinated in the headquarters office in Mexico City but the work is decentralized into regional offices in each of the state capitals. The state and Federal Government jointly contribute finances for its operation. This Division provides the mechanism for utilization of any health activity throughout Mexico for the purpose of training or investigation by any agency such as the School of Hygiene, Institute of Tropical Medicine, etc.

#### *Dirección de Cooperación Interamericana de Salubridad Pública*

In 1943 there was established as an integral part of the Secretaría de Salubridad y Asistencia a cooperative inter-American public health service known as the Dirección de Cooperación Interamericana de Salubridad Pública. It is jointly financed by the Secretaría de Salubridad y Asistencia and the Institute of Inter-American Affairs (Office of Inter-American Affairs). It also receives personnel and services from the two institutions.

This program has devoted principal attention to construction of Public Water Supplies and Sewerage Systems in smaller cities, and health center buildings. It has also supported other types of Public Health Activities. In 1942 the Pan American Sanitary Bureau with financial support from the Office of the Coordinator of Inter-American Affairs, collaborated with the Secretaría de Salubridad y Asistencia in the initiation of a venereal disease control program along the northern border of Mexico. Subsequently a demonstration and training venereal disease control center was established in Mexico City. The continued operation of these projects is being financed by the Secretaría de Salubridad y Asistencia and the Dirección de Cooperación Interamericana de Salubridad Pública. Similarly a demonstration tuberculosis control program is being initiated along the northern border of Mexico. Certain projects for expansion of activities in the School of Hygiene and for research in the Institute of Tropical Medicine are being supported by the Dirección de Cooperación Interamericana de Salubridad Pública, and also in Pinta and Onchocerciasis. Training of personnel is of primary concern.

#### *Other Activities of Secretaría*

Space does not permit a summary of all the activities of the Secretaría de Salubridad y Asistencia, which provide opportunities for training and research in tropical medicine and public health. Mention must be made of the program of maternal and child hygiene which is a part of every health center activity. Also a very ambitious program for the control of tuberculosis is under way.

Within a few months three additional modern sanatoria will be in service. Considerable attention is being devoted to the problem of health education.

#### *Availability of Facilities to other Countries*

Mexico, through its Secretaría de Salubridad y Asistencia, is happy to make available her facilities for training and research to students or investigators of other countries. She will be pleased to share her experience in public health or tropical medicine with others and will hope to gain suggestions and new ideas thereby. In the post war period the return of millions of soldiers from tropical service to temperate or semitemperate climates constitutes a problem of the first magnitude in tropical medicine and in public health. The study of these problems in their native environment should prove helpful.

#### SUMMARY

The present war has focused attention upon Tropical Medicine and Tropical Public Health. The Secretaría de Salubridad y Asistencia of Mexico provides unusual opportunities for training and research in these specialities. The Instituto de Salubridad y Enfermedades Tropicales is the research center in tropical medicine. Field stations for the study of onchocerciasis exist in Huixtla, Chis.; for pinta at Arcelia, Gro., and for general tropical medicine in Boca del Río, Ver. A broad program in nutrition centers around the Instituto de Nutrición, and the Hospital for Nutritional Diseases. The control of malaria and other parasitic infections is receiving intensive attention, as well as typhus, pinta, and leprosy. Other laboratory facilities exist in the Laboratorio Central and Instituto de Higiene, as well as clinical facilities in her many hospitals.

The Escuela de Salubridad e Higiene offers a complete course of instruction for Public Health Physicians, nurses and sanitary inspectors, including field work. Several well organized Health Centers serve as training stations for Public Health Personnel. Opportunities are available for specialized training as for example maternal and child hygiene, tuberculosis or venereal disease control.

While these facilities were organized primarily for the training of Mexican personnel, they are at the disposition of other countries. Many of the staff of the Secretaría de Salubridad y Asistencia are bilingual.

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# HEAT RASH AS A PROBLEM IN THE NAVAL SERVICE<sup>1</sup>

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Heat rash, also known as prickly heat, miliaria rubra and Lichen tropicus, is a problem of little concern in civil medical practice. While the Pacific fleet was operating in tropical waters, however, this condition consumed a good deal of a medical officer's time aboard these ships. The cause of miliaria in the naval service is excessive heat. The equipment installed aboard men-of-war for purposes of ventilation is placed there at the expense, in terms of tonnage, of military equipment. It is, therefore, not practical to have ideal air conditions in ships' living spaces. With mechanical ventilation of the type currently employed, the compartment air is cooled by dilution with weather air. The air temperature of a space aboard ship is never equal to that "topside." The dry bulb temperature below decks is always 7 to 10 degrees F. above that of the weather temperature. During fleet operations in the Pacific war, ships were forced to spend long periods at General Quarters at which time no ventilation equipment was in operation. At sunset ships were darkened and at this time all weather accesses were closed. These tactical necessities contributed greatly to the heat load developed in living and working spaces. On a modern well ventilated battleship 70 per cent of the crew were sleeping in spaces in which the temperatures were sufficiently high to produce heat rash.

The pricking, burning and itching of the skin which accompanies this condition causes insomnia and is deleterious to the ship's company morale and efficiency. The modern "blue jacket" cannot be expected to accept a disability merely as a hardship of sea going life. A considerable number of man hours are lost to the service when men report several times daily to the sick bay to have lotions applied to their itching skins. On one ship an average of 135 of the crew, numbering 2700, answered sick call daily. Approximately 40 per cent of those seen at sick call complained of heat rash. Heat rash and its complications represented 37.4 per cent of the skin cases seen at a medical activity on Guam.

Medical officers in the Navy have long been interested in the adverse effects of exposure to heat. In several recent experiments at the Naval Medical Research Institute, heat rash was studied as one of these effects.

In the first of these experiments ten volunteers designated as the "Hot Group" were selected at random as subjects to live under conditions simulating those aboard ship in the tropics. This group worked seven hours per day in a room maintained at 108°F. dry bulb and 83°F. wet bulb, an effective temperature of 90°, and spent the remaining 17 hours of the day in another room maintained

<sup>1</sup>Read at the Annual Meeting of the American Society of Tropical Medicine, at Cincinnati, Ohio, November 14th, 1945.

The material in this article should be construed only as the personal opinion of the writer and not as representing the opinions of the Navy Department officially.

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at 95°F. dry bulb and 83°F. wet bulb, an effective temperature of 87°. The work for each man consisted of walking on a treadmill. A similar group of ten volunteers known as the "Cool Group" was selected, for the second part of the experiment. They likewise worked seven hours a day in the hot treadmill room, but the remaining 17 hours were spent in an environment of 85°F. dry bulb and 71°F. wet bulb, an effective temperature of 78°. Each group continued its respective program for ten days without interruption.

All the subjects in the "Hot Group" developed heat rash, an average of 39 per cent of the skin surface of each subject being involved. Half of the subjects in this group complained that they were unable to sleep due to the discomfort produced by the rash. Only one subject in the "Cool Group" developed heat rash and in that case only 15 per cent of the body surface was involved.

Twelve seamen, all 18 years of age, volunteered for the second experiment. One group was again designated the "Hot Group" and the other the "Cool Group." The men wore shorts throughout the experiment. Three psychrometric rooms were used in the experiment. One was designated the "hot quarters," one the "cool quarters," and the third the treadmill room. All three psychrometric rooms were set at 80°F. dry bulb and 70°F. wet bulb, when the experiment was started. This cool condition was maintained for the first eight days. On the morning of the ninth day the temperature in the hot quarters was elevated to 90° dry bulb and 85° wet bulb, an effective temperature of 85°, and the treadmill room was elevated to 108° dry bulb and 83° wet bulb, an effective temperature of 90°. The cool quarters were continued at 80° dry bulb and 70° wet bulb. The "Hot Group" spent three hours a day in the treadmill room and the remaining 21 hours in the hot quarters. The "Cool Group" spent three hours a day in the treadmill room, nine hours a day in the hot quarters and the remaining 12 hours in the cool quarters. These conditions were continued for 30 days.

All but one subject in the hot group developed miliaria. The rash appeared as early as the third day of residence in the hot atmosphere. The rash spread rapidly and reached maximum severity on the ninth to thirteenth day. The cool group, it can be seen, was spared this ordeal. Lowering of the temperature at the conclusion of the experiment was accompanied by a complete regression of the rash.

The obvious conclusion that can be drawn from these experiments is that spending as little as 12 hours a day in an atmosphere in which one does not sweat at rest (78° effective) will prevent the occurrence of miliaria.

Further studies in this field were carried out. The findings in these experiments are as yet unpublished. The subjects were exposed continuously to an effective temperature of 89.6°, 97.8° dry bulb and 86° wet bulb in these experiments. The erythematous papular and papulo-vesicular rash appeared suddenly on the third day of the experiment.

The investigators found that there was no correlation between the pH of the sweat, sweating rates or complexion and the incidence or severity of heat rash.

Formalin electro-phoresis, taking or abstaining from shower baths and pressure bandages over certain areas did not affect the course of the disease.

Exposure to ultraviolet radiation was found to ameliorate the symptoms in some cases of miliaria but in others increased its severity. The conditions accounting for this discrepancy are not clearly understood.

The pathological changes found by biopsy in one case of experimentally produced heat rash were as follows: (1) The epidermis of the heat rash area is hyperplastic, (2) a blister forms in the stratum lucidum and is unrelated to the sweat gland or hair follicles and (3) the dermis is edematous and gives every indication of a dermatitis. Some of the inflammatory cells infiltrate the epidermis.

Heat rash represents a serious problem aboard ship in tropical waters. It can be prevented by short periods of cooling. During this war certain critical spaces such as sick bays, have been air cooled. The U. S. Navy is the only one of the world's navies that has taken steps to protect men from hot environments aboard its ships.

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## MILITARY AMEBIASIS

### OBSERVATIONS ON THE COMPLETE COURSES OF FORTY CASES, WITH RESULTS OF TREATMENT

EDDY D. PALMER<sup>1</sup>

Clinical observations on war-time military amebiasis are hampered by the constant necessity for evacuating the patient. Important information covering phases of the course of the disease in World War II has been collected in Army medical installations along the chain of evacuation (Edson, Ingegno and D'Albora, 1945), but final evaluation, especially in regard to the long-range results of treatment, has not yet been made. This paper is a report on the complete course of the disease, with exposition of the results of treatment, in Army amebiasis patients who had reached the terminus of the chain of evacuation, a general hospital in the Zone of the Interior, and who were eventually termed cured and discharged from medical observation.

#### MATERIAL

Observations on 40 consecutive soldiers who had been diagnosed here or at previous medical installations as amebiasis in any of its forms, and whose clinical records, past and present, were complete in detail regarding this infection, are reported. There was no selection of cases provided clinical information was complete and concurrent disease did not confuse the amebiasis picture. There were 37 white and three colored patients. There was one female. The ages varied from 20 to 45, with an average of 33.9 years.

Amebiasis had been first diagnosed and treated in each case while the patient was in military service. Two had served in the Zone of the Interior only, and six were first diagnosed while prisoners of the Japanese in the Philippines. Five had served in the China-Burma theater, 25 in the south-west Pacific, one in the European theater, and one in north Africa.

Eleven patients admitted having eaten large quantities of raw native vegetables overseas, and 10 believed their drinking water was probably unsafe at times.

Thirteen patients had been evacuated because of their amebiasis and had no other demonstrable disease processes, except in some cases other intestinal parasites. The other 27 patients had a variety of diseases in addition to amebiasis during their present course of hospitalizations, namely, lichen planus, tropical ulcer, convalescent beriberi, convalescent scurvy, relapsing tertian malaria, subclinical schistosomiasis japonica, gonorrhea, latent syphilis, duodenal ulcer, chronic sinusitis, and asthma.

Twenty-seven of the 40 patients harbored intestinal parasites other than *Endamoeba histolytica* (table 1). There was a total of 53 infections with 10 other species. *Endamoeba coli* and hookworm infections were most prevalent.

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All of these infections were subclinical, as shown by the eventual clearing of gastrointestinal symptoms and signs following cure of amebiasis, in spite of persistence of these other infections. Treatment was later given for the other pathogenic species.

#### TYPES OF AMEBIASIS REPRESENTED

Of the 40 patients, 28 had uncomplicated symptomatic amebic colitis, three were asymptomatic "carriers," four had at some time during their courses developed amebic hepatitis, three had uncomplicated amebic liver abscesses, one had amebic liver abscess complicated by diaphragmatic perforation and amebic empyema, and one had multiple amebomas of the transverse colon. Three cases of symptomatic amebic colitis eventually developed typical chronic

TABLE 1  
*Other species of intestinal parasites in 40 amebiasis patients*

PARASITE	NUMBER OF PATIENTS
None.....	13
<i>Endolimax nana</i> .....	4
<i>Iodamoeba bütschlii</i> .....	5
<i>Endamoeba coli</i> .....	11
<i>Trichomonas hominis</i> .....	4
<i>Giardia lamblia</i> .....	2
<i>Schistosoma japonicum</i> .....	1
<i>Trichocephalus trichiuris</i> .....	5
<i>Ascaris lumbricoides</i> .....	8
Hookworm.....	10
<i>Strongyloides stercoralis</i> .....	3
Total number of infections.....	53

idiopathic ulcerative colitis (Bargen's type I) while under observation following cure of amebiasis.

#### PERIOD OF HOSPITALIZATION

The period of hospital study, and incidentally time lost to the Army, of the patients considered here was lengthy. The six patients who were prisoners of the Japanese for long periods did not receive proper anti-amebic treatment, and, therefore, their periods of hospitalization are not considered. Three asymptomatic "carriers" were hospitalized for an average of 82 days because of their infections. The other 31 patients were studied for an average of 12.4 months for their amebiasis alone, excluding hospitalization for complicating idiopathic ulcerative colitis (table 2). Seven were hospitalized 18 to 22 months, eight 12 to 17 months, eleven 8 to 11 months, and five 4 to 7 months. Administrative procedures tended to prolong hospitalization in some cases, but this factor could not be satisfactorily measured and excluded.

## DATA SOURCES

The information reported here was obtained from the records of the patients' current series of hospitalizations, including those compiled at this installation. The previous medical records of the 40 cases were complete in detail regarding clinical findings and treatment measures, and these data are reported. In many cases a minimum of laboratory, X-ray, EKG and sigmoidoscopic studies was done overseas. Because the laboratory techniques used in other installations for stool examination are not known, previous stool reports, unless positive, were felt to be potentially misleading and are not included. Certain other data are not reported when it seemed that they could have been influenced by other disease processes present.

## CLINICAL FINDINGS

*Onset.* The onset of the clinical disease was sudden in 28 cases and gradual in nine. The former group included the eight cases of hepatitis and liver abscess, and the latter the case of colon amebomas. Three patients were consistently asymptomatic.

TABLE 2

*Length of period of hospitalization for amebic infection alone in 40 amebiasis patients*

NUMBER OF PATIENTS	MONTHS OF HOSPITALIZATION
3 ("carriers")	2
5	4-7
11	8-11
8	12-17
7	18-22
(6 (POW)	25-48)

*Presenting symptoms.* The presenting symptoms of two of the four liver abscess patients were fever and steady right upper quadrant abdominal pain. Those of the other 33 symptomatic patients were abdominal cramps and diarrhea, 13 with gross blood and 20 without. Evidences of hepatitis or liver abscess in six patients developed two to 22 months after the establishment of clinical colitis.

*Symptoms on relapse.* Each patient who had one or more post-treatment relapses stated that the type of onset and presenting symptoms each time were identical to those of the initial attack. Most felt that the symptoms were most severe during the first attack.

*Remissions and exacerbations.* There were no spontaneous remissions of symptoms in the hepatitis, liver abscess and ameboma patients. Half of the 28 uncomplicated symptomatic colitis patients had no partial or complete remissions until therapy was instituted. The other 14 had recurring spontaneous remissions lasting two to 30 days, punctuated with exacerbations of about the same duration. In each case any tendency to remission and exacerbation was remarkably constant during successive relapses.

*Hepatitis patients (four).* During the active phase of the liver complication, each of the hepatitis patients complained of severe malaise, anorexia, right upper quadrant pain, jaundice, crampy lower abdominal pain, tenesmus, and 11 to 60 watery stools with gross melena per day. One patient had persistent nausea, vomiting and stomach gas.

*Uncomplicated liver abscess cases (three).* The symptoms of the patients with liver abscess differed from those of the hepatitis patients in that they all had fever, while only two had brief periods of jaundice and only one had crampy lower abdominal pain, tenesmus, diarrhea and gross melena. Two had brief periods of nausea and vomiting.

Each case showed a patternless erratic spiking fever from 99 to about 103.8°F., which in each case returned to normal on the second day of emetine therapy, to recur unless cured within four weeks of the completion of the emetine course. The livers were found to be 1 to 5 cm. below the costal margin at the time diagnosis was made or relapse detected. No abscess was palpable. Two abscesses were demonstrated at the superior surface of the right lobe by X-ray and one was found only by the exploring needle at about the center of the right lobe. Two sigmoidoscopic examinations on each case before cure failed to reveal pathology, although material aspirated from the normal appearing rectal mucosa in one instance was positive for *E. histolytica*. Tenderness of the cecum and sigmoid was found in one patient. There was no anemia. Leukocytosis of 12,700 to 17,200 was consistently present prior to therapy and during relapse.

In the one liver abscess patient in whom thorough laboratory evaluation of the liver function was made there were no abnormal findings. The bromsulphalein, Hanger, and oral hippuric acid tests, and van den Bergh, icteric index and blood protein determinations were normal during the active phase and after cure.

*Liver abscess complicated by diaphragmatic perforation (one case).* This patient developed an acute bloody diarrhea and was hospitalized and treated for amebic colitis, although several stool examinations failed to reveal the organisms. There were no subjective or objective findings at the time to indicate liver involvement. Six weeks after the onset of the colitis, the sudden development of severe right pleuritic pain and dyspnea with signs of fluid at the base led to pleural aspiration. Material from the pleural space revealed for the first time *E. histolytica*.

*Uncomplicated symptomatic colitis (28 cases).* Of the 28 patients, 17 had poor appetites, occasional nausea and stomach gas. Five had brief periods of vomiting associated with crampy upper abdominal pain. Eighteen had gross melena for periods longer than two weeks. All had lower abdominal crampy pain. Stools were largely mucus and blood for long periods in two, and watery or mushy in the others. Sixteen complained of persistent tenesmus. The patient's estimate of the average number of stools per day during the entire course of the active disease ranged from five to 60, with a mean for all cases of 12.

During the active stages, none had fever, objective evidence of weight loss which could be ascribed to amebiasis, anemia, or laboratory evidence of im-

paired liver function. The liver was recorded as enlarged 1 to 2 cm. below the costal margin in four cases. The sigmoid was tender in 19 cases and the cecum in 16. Twenty of the patients were sigmoidoscoped on at least one occasion (average 2.1) during the active stage, and typical amebic pathology was found in 11. In eight of 15 cases rectal aspirations were positive for *E. histolytica*, and in two of these cases specific diagnosis was made in no other way. In eight cases there was a low-grade leukocytosis which could be attributed to no other cause. Microscopic melena was universally present at some time during the active disease, and often persistently. Of 13 patients submitted to barium enema studies before treatment, the report for four showed the changes described by Golden and Ducharme (1945) as suggestive of amebiasis in the cecum, and for another five inflammatory spasm in the sigmoid alone, compatible with amebic colitis (Druckman and Schorr, 1945).

*Multiple amebomas of the transverse colon (one case).* The subjective and objective findings in this patient, who was under continuous medical observation for 23 months, were similar to those in the cases of symptomatic colitis. Exceptions were a persistent leukocytosis of 13,200 to 14,800, a sedimentation rate of 45 to 47 mm. per hour (Westergren), and the finding of tumors by barium enema X-ray; all persisted until the completion of the curative course of therapy. The diarrhea was moderately severe and rather tenacious. There was considerable gross melena during the periods of more severe diarrhea. Sigmoidoscopic examinations showed a typical mild amebic ulcerative proctitis involving the regions of the valves, but organisms were not recovered by mucosal aspiration. There was no fever, anemia, or clinical or laboratory evidence of hepatic involvement.

The tumors were not palpable and were not within reach of the sigmoidoscope. Serial barium enemas, eight in all over a 20 month period, showed the size and configuration of the tumors to be static until the beginning of regression following successful treatment. There were two tumors, in the transverse colon, 8 cm. apart; the remainder of the colon and cecum was normal, with filling of the appendix and barium reflux into the terminal ileum. The tumors were similar in size and shape, the areas of constriction measuring 8 cm. and the lumen of the bowel being constricted to about two-thirds of normal by each. The important radiologic features, observed consistently, were the gradual transition from normal bowel to tumor and the absence of mucosal irregularity; the absence of overhanging edges was remarked on several times. Although the roentgenologists at three installations mentioned the possibility of malignancy, the smooth sloping transition to tumor tissue was the determining factor in the general impression of benignancy.

*Idiopathic ulcerative colitis.* Three cases of symptomatic amebic colitis developed typical idiopathic chronic ulcerative colitis (Bargen's type I) following cure. This developed in each case during post-treatment observation, i.e., within two months of active amebiasis. In two patients the sigmoidoscopic picture had returned to normal in the interim; in the other at the time the onset of the acute stage of ulcerative colitis was detected there were circumscribed patches of mild residual hyperemia in the region of the former amebic rectal

ulcers. The patients were observed for periods of 11, 12, and 21 months respectively following the establishment of the diagnosis of ulcerative colitis. The onset in all cases was acute, with typical rectal and lower sigmoid changes consisting of generalized intense hyperemia and edema, with closely spaced pin-head sized superficial abscesses and erosions, friability, easy bleeding and copious purulent exudate. Stools and rectal aspirations were consistently negative to microscopic examination and culture. X-ray studies and the eventual course in each case were consistent with those usually associated with the disease. One patient eventually came to pancolectomy.

#### TREATMENT AND RESULTS

*Criteria of cure.* All patients, including those who had been considered cured at other installations, were submitted to the following procedures at this hospital, whatever the type of amebiasis, before being considered free of infection. Each patient following cure was studied a minimum of two months (average 2.5 months) of continuous hospitalization.

Clinical criteria included absence of fever, leukocytosis, hepatomegaly, and rectal and lower sigmoid pathology by a minimum of two (average 2.5) sigmoidoscopic examinations. Continuation of colon tenderness and diarrhea was not considered necessarily to be evidence of continuation of active amebiasis; it was commonly observed that amebic colitis patients continued to have diarrhea for periods of about two months following cure, their bowel movements gradually reverting to normal spontaneously. Actually in patients who had had simple amebic colitis, no changes in the clinical picture, except the sigmoidoscopic findings, were necessarily helpful in estimating the effectiveness of treatment.

Laboratory criteria included a minimum of two (average 2.5) negative sigmoidoscopic rectal aspirations and a minimum of six (average 9.9) negative stools. The material obtained by rectal aspiration was examined directly under the warm-stage. Each stool was examined by direct smear, the modified zinc sulfate flotation concentration technic of Otto, Hewitt and Strahan (1941), and concentration by centrifugalization technic (Craig and Faust, 1940). The findings of Sawitz and Faust (1942) and Sawitz and Hammerstrom (1943) indicate that these were exceptionally rigid criteria.

The case of amebomas of the colon was considered cured after meeting the above criteria and after X-ray studies had shown complete regression of the tumors.

*Number of courses of treatment.* Of the 40 cases, two lost their infections spontaneously without treatment, 22 were cured by the first course of treatment, nine only after two courses, six after three, and one after four (table 3). The asymptomatic group appeared to be the easiest to cure and the liver abscess cases the most difficult. Eleven of the 28 symptomatic colitis cases required more than one course of treatment.

*Hepatitis.* Of the four cases of amebic hepatitis, three were cured following one course of treatment which included emetine (table 4). The other patient relapsed four months after apparent cure with carbarsone; treatment was then

resumed, with subsequent cure. This latter case received no emetine or iodides.

TABLE 3

*Number of courses of treatment required to cure 40 cases of amebiasis*

TYPE OF AMEBIASIS	NUMBER OF PATIENTS				
	Number of courses of treatment				
	0	1	2	3	4
Asymptomatic ("carriers").....	1	2			
Uncomplicated colitis.....	1	16	8	3	
Amebomas of colon.....				1	
Hepatitis.....		3	1		
Uncomplicated liver abscess.....		1		1	1
Liver abscess with diaphragmatic perforation.....				1	
Totals.....	2	22	9	6	1

TABLE 4

*Summary of treatment and results in four cases of amebic hepatitis*

CASE	EMETINE	CARBARSONE	DIDOQUIN	CHINIOFON	CLINICAL RELAPSE
	grams	grams	grams	grams	months
1	0.46	7			0
2	0.40	20	39.1		0
3	1.00	15.75		6	0
4 (1st)		3.5			4
(2nd)		7			0

TABLE 5

*Summary of treatment and results in three cases of uncomplicated amebic liver abscess*

CASE	SURGICAL PROCEDURE	EMETINE	CARBARSONE	DIDOQUIN	CLINICAL RELAPSE
		grams	grams	grams	months
1	Open drainage and aspiration	1.1		7.8	0
2 (1st)		0.55	7.5	19.6	9
(2nd)		0.59	10.5	13.7	1
(3rd)		0.33	6	87.2	0
3 (1st)	Open drainage				2
(2nd)	Open drainage				1
(3rd)		1.17			2
(4th)		1.17	7.5	13.7	0

*Uncomplicated liver abscess.* The three cases of uncomplicated liver abscess required one, three and four courses of treatment respectively for cure (table 5).



One was cured without surgical procedures, one had had two open drainages with relapse following each before cure, and one had open drainage with aspiration four days later. All had large doses of emetine.

*Liver abscess complicated by diaphragmatic perforation.* The patient who developed diaphragmatic perforation from liver abscess had been treated for colitis with a course of emetine 0.78 gm. and carbarsone 18 gm., completed about one week prior to perforation. Following perforation and the recovery of amebae from the pleural fluid, he was given an additional 1.43 gm. of emetine and 19.6 gm. of diodoquin in two courses, with a single aspiration of the liver abscess and an additional aspiration of the pleural space. No local therapy was used. No hepato-bronchial fistula developed. The patient recovered without clinical or laboratory relapse.

*Multiple amebomas of the transverse colon.* This patient received three courses of treatment, which included emetine, carbarsone, diodoquin and enemas of chiniofon, over a 16 month period before cure (table 6). No surgery was attempted. The tumors were discovered at the time of the first treatment for colitis. The subsequent two courses were instituted when stool relapse was

TABLE 6

*Summary of treatment and results in one case of multiple amebomas of the transverse colon*

COURSE	EMETINE	CARBARSONE	DIDOQUIN	CHINIOFON ENEMAS	STOOL RELAPSE
	grams	grams	grams	grams	months
1st	1.0	20		5 (200)	4
2nd	1.0	20			8
3rd	0.26	3	13.65		0

discovered following return of colitis symptoms. Barium enemas showed no regression of the tumors until the completion of the successful third course, when they gradually disappeared over a two month period.

*Uncomplicated amebic colitis.* The 29 treated cases of uncomplicated colitis received an aggregate of 43 courses of treatment, i.e., 14 were not curative (table 7). It is to be noted that the infections in two patients, who had been shown by stool examination to have amebiasis, cleared spontaneously without treatment, as proven by the stated criteria, with 12 negative stool examinations each.

A great variety of drug regimes was used in the various medical installations handling these cases. In five courses only one drug was used, and in 14 two. The other 24 courses combined emetine, carbarsone, and diodoquin in various doses. Chiniofon was used on one occasion.

Among the 14 unsuccessful courses of treatment in this group, eight were followed by stool relapse in from two to four months after the completion of treatment. Three were stool positive sooner and three later, with extremes of 10 days and 11 months. Clinical relapse occurred from three weeks to six months following treatment.

Table 7 permits an important observation: in this series the results of treatment could not be predicted from the size of drug dose. Ten of the 14 treatment failures followed courses combining doses of arsenical and iodide which were small as compared with doses which proved to be curative, but eight such courses were successful. The other four treatment failures followed courses

TABLE 7

*Summary of treatment courses and results in 31 cases of uncomplicated symptomatic and asymptomatic amebic colitis*

TREATMENT COURSE				NUMBER OF INFECTIONS TREATED	STOOL RELAPSE (MONTHS)					
Emetine	Carbar- sone	Diodo- quin	Chiniofon		0	0-1	1	2-4	5-10	11
grams	grams	grams	grams							
0	0	0	0	2	2					
	7.5			2				2		
	15			2	1			1		
		13.72		1				1		
0.26	3			1						1
0.39	7			2	1					1
0.65	7.5			1					1	
0.85	11.25			1	1					
0.78	22.5			2	2					
0.46	300			1	1					
0.65		17.94		2	1			1		
	5.25	4.55		1	1					
	8	13.72		2	2					
		27.04	14	1	1					
0.26	3	1.66		1		1				
0.20	3	2.50		1	1					
0.26	3	7.90		1	1					
0.13	4.25	13.72		1	1					
0.13	6	11.65		1	1					
0.20	5.25	13.72		1	1					
0.33	6	11.65		1	1					
0.52	7.5	1.66		1				1		
0.52	6	13.72		3	3					
0.39	7	23.25		4	2		2			
0.65	5	3.95		1	1					
0.46	10	19.55		2				2		
0.52	7.5	31.20		1	1					
0.78	6	7.90		1	1					
0.46	7.5	37.44		1	1					
0.91	7	13.72		1	1					
1.37	8.5	13.72		1	1					
0.39	4.5	56.99		1	1					

which combined larger doses of arsenical and iodide than those which were curative in 13 cases.

The inclusion of and dose of emetine in the regime bore no direct relation to the effectiveness of the therapy, although this fact is somewhat obscured by the tendency to include relatively large doses of emetine in regimes which in-

clude large doses of the other drugs. The 29 successful regimes employed an average of 0.42 gm. of emetine while the 14 unsuccessful ones employed an average of 0.31 gm.

*Toxic effects of emetine.* Of the 33 cases treated with emetine (maximum total dose 2.34 gm., average 0.77 gm.), one developed a sterile local abscess after a course of 0.39 gm., and one a polyneuritis of two months duration following 0.46 gm. There were no other clinical evidences of untoward reaction. The blood pressure and cardiac sounds were followed closely in each case, even under apparently difficult overseas conditions, without significant change. Ten patients were followed during one course of emetine each by serial electrocardiograms (average 3.6, in addition to pretreatment base-line records); no changes were found in six patients, while in four there was inconstant depression of the T-waves, with reversion to pretreatment amplitudes following cessation of emetine.

#### DISCUSSION

Ochsner and DeBakey (1939a) suggested the term "ameboma" for chronic granulomatous tumors of the large bowel of amebic etiology. This lesion is perhaps rather common. Its usual site is the rectum, and it usually is single. Hu (1937), Donald and Brown (1940), D'Antoni (1943) and Lindskog and Walters (1946) have reported a total of 11 cases, all of which were single and 10 of which were in the rectum. The amebomas in D'Antoni's three cases disappeared following medical treatment. The case of Lindskog and Walters required resection of the lesion after four unsuccessful courses of emetine and one of carbarsone (doses not stated); the lesion was in the ileo-cecal region, measured 4 cm. in diameter, and showed no amebae at the time of surgery.

The case reported in the present series is interesting because of the opportunity afforded to observe the tumors radiologically over a 20 month period. Their static nature, especially during successive non-curative courses of antiamebic treatment, is notable. It was only after the amebae were permanently eliminated that the tumors began to clear. The regression was eventually complete. Although there were many misgivings on the parts of several observers during the course of the patient's illness, it was gratifying that faith in the effectiveness of non-surgical treatment was eventually realized.

The continuation of diarrhea following cured amebic colitis—cured according to acceptable criteria—is a curious phase of the natural history of the disease. It has been commented upon seldom (D'Antoni, 1942), but has so impressed observers in at least two Army tropical diseases centers that patients are routinely warned prior to treatment to anticipate this discouraging sequela. When emetine is part of the treatment regime, the diarrhea may be expected to cease rather quickly, to return about a week after the drug is discontinued, and to persist with gradually decreasing severity without further treatment for about two months. When iodides and/or arsenicals are used alone, the diarrhea may be expected to persist without interruption throughout therapy and to decline slowly thereafter. The most significant change in the character of the stool

upon the elimination of the infection is the disappearance of gross and occult blood. This is consistent with the clearing of the ulcers as observed through the sigmoidoscope. But there is no observable persistent pathology to explain the continuation of the diarrhea—this must be a matter of functional hypermotility. Numerous follow-up sigmoidoscopies in several patients during this period of unexplained post-treatment diarrhea have revealed no pathology—no hyperemia, excess mucus, edema, or residual abnormal changes from healed ulcers.

Craig (1944) has observed persistent diarrhea after cure of chronic amebic dysentery, but only in those patients in whom extensive ulceration has occurred with resulting irreversible enormous thickening of the bowel wall, observed by sigmoidoscopy and at autopsy. He distinguishes this sequela from chronic idiopathic ulcerative colitis.

The three cases of idiopathic ulcerative colitis reported here were observed from their inception. There was nothing peculiar about them except the circumstances immediately preceding their onsets—the recently extant amebic colitis in each case. No acceptable causal relationship between the two diseases could be established, of course. It is clear that they did not represent the type of chronic post-treatment colitis described by Craig (l.c.), because the bowel had in each case returned to essential normality before the onset of the second disease. It seems likely for this very reason that the diseases were in no way related. Other investigators have not given a definite answer to the question of possible relationship, and a search of the literature brought to light only speculations. In spite of lack of controlled clinical data, however, one may well respect the impressions of the many competent observers who are almost universally agreed that there is no causal relationship between amebiasis and chronic idiopathic ulcerative colitis. Statements like the following (Silverman, 1945) are frequently found: "After studying more than a thousand cases of acute and chronic . . . dysentery over a period of years I believe that neither *Bacterium dysenteriae* nor *Endameba* (sic) *histolytica* has anything to do with the cause of chronic ulcerative colitis."

It was a matter of considerable interest to note that in each amebic colitis patient symptoms on relapse were qualitatively remarkably similar to those of the initial pre-treatment phase. No comment on the matter has been found in the literature. It would indicate, perhaps, that a rather precise and static pathologic relationship exists between host and parasite, and that the interrelationship of the strain individualities of both play a significant part in the determination of the clinical manifestations.

In attempting to evaluate the effectiveness of treatment in amebiasis, as in other infectious diseases which confer little or no immunity, the greatest source of potential error and quasi-scientific conclusion is the difficulty in differentiating relapse from reinfection. The difficulty is enhanced when considering soldiers in war-time because the heterogeneity of overseas geographic conditions plus the constant movement of troops renders the use of control primary-attack rates unreliable. The issue is further clouded by the asymptomatic "carrier" stage of amebiasis.

It was felt, however, that a reliable estimate of the situation could be made in this series. Because the patients had been protected from reinfection by continuous hospitalization without overseas furlough since the onset of the disease, and because the period between treatment and relapse had been no longer than 11 months in any patient, it was thought that the "relapses" reported here were probably true relapses rather than reinfections. The 11 month maximum was taken arbitrarily, with the realization that the average incubation period in acute amebic colitis is probably only about two months and the prepatent period about nine days (Walker and Sellards, 1913).

Emetine 0.65 gm. over a 10 day period is the treatment of choice in amebic hepatitis (Craig, 1944). This authority states (l.c.), "... emetine is an absolute specific in the cure of amebic hepatitis and if this condition is recognized a course of treatment with this drug is always successful in relieving the symptoms and preventing abscess formation." Sodeman and Lewis (1945), reporting 33 cases, have been only two of several investigators to add confirmation to this statement. The fact that a case in the present series was cured with carbarsone alone, and that D'Antoni (1946) has noted disappearance of liver pain and tenderness in several amebiasis patients during treatment with diodoquin, constitute no recommendation that oral amebicides be relied on routinely in this complication of amebiasis. However, should emetine be contraindicated in a patient for any of several reasons, one might proceed with iodides and arsenicals with some confidence in their effectiveness. D'Antoni (l.c.) comments that, since diodoquin is not absorbed, its ability to render some cases of amebic hepatitis asymptomatic might indicate that *E. histolytica* is not always present in the liver in this complication.

The treatment of amebic liver abscess has been so completely and thoroughly described and discussed in the past (Ochsner and DeBakey, 1935; 1939a; 1939b; 1943; Ochsner, DeBakey and Murray, 1939) that no elaboration on this small series is warranted. Perhaps, however, it is permissible to note here without further discussion that one case of abscess was cured without surgical interference, and that one case relapsed twice following open drainage but was cured later by medical management alone. All abscess cases were handled in rather unorthodox fashions.

The variously conceived courses of treatment which were used, especially in the colitis patients (table 7), with their many different dosages of several standard drugs, might have offered an unusual opportunity for comparative study, were the series larger. There must be in such a series a certain amount of inconsistency between the size of drug dosage and its effectiveness, by reason of biologic and pathologic variation in the strains of both the organism and the host. In a small series this feature must preclude statistical evaluation.

One important observation, however, was made. The often emphasized impotency of emetine in influencing the eradication of *E. histolytica* from bowel lesions (Manson-Bâhr, 1941; Craig, 1944), was confirmed, but a similar but variable impotency, or at least an unpredictable variation, in the effectiveness of the oral amebicides, not anticipated from mere strain variation or from the

experiences of other workers, was also found. It was a therapeutic disappointment to note the erratic response to various doses of the various drugs. Thus in two instances courses of emetine 0.46 gm., carbarsone 10 gm., and diodoquin 19.55 gm. were not curative, while a course of emetine 0.20 gm., carbarsone 3 gm., and diodoquin 2.50 gm. was. Other examples are evident in the table. The matter is further confused—or possibly clarified—by the spontaneous disappearance of the infection without treatment in two cases.

Spontaneous disappearance of *E. histolytica* following the establishment of a well defined infection has been commented on by Craig (1944), who has seen several cases. Statistics on the matter have not been published. The two cases (5 per cent of the series) reported here were studied carefully, and the existence of amebiasis at one point and its absence at another without treatment were confirmed according to rigid criteria. The manner of selecting cases should not necessarily have influenced the incidence here, but the series is small.

The potential dangers of emetine therapy were brought out here. The insidious nature of these is reflected in the fact that both patients who had untoward reactions had not received large doses of the drug. There would be, of course, no expected relationship between size of dose and development of a subcutaneous abscess, but the polyneuritis would seem to be a different matter. One notes, in this connection, that Berkman and Barger (1942) reported a case of amebiasis who received a total of 12.61 gm. of the drug over a period of several years without recognized toxic effects, while Brown (1935) collected cases in adults in whom death followed the administration of 1.28, 1.74, 1.07, 1.44, 1.52, 1.04, 1.08, 1.88, and 0.48 gm. It is notable that 10 patients in the present series treated with emetine were closely supervised regarding the most serious potential complication, myocardial damage, and no abnormalities or change in status were found, other than insignificant changes in the T-waves of the electrocardiogram.

#### SUMMARY

1. Observations on the complete courses of 40 cases of amebiasis among military personnel are reported. There were three asymptomatic "carrier" cases, 28 patients with symptomatic amebic colitis, four with amebic hepatitis, three with hepatic abscess, one with hepatic abscess complicated by diaphragmatic perforation, and one with multiple amebomas of the transverse colon. They were studied under continuous hospitalization for average periods of more than a year.

2. The clinical findings in general did not vary remarkably from those usually ascribed to the disease. Two features were impressive:

a. The patients' remarkable qualitative constancy of symptoms, including tendency to remission and exacerbation, before treatment and during each relapse.

b. The persistence of colon tenderness and bloodless diarrhea, with negative sigmoidoscopic picture, in cured amebic colitis patients for about two months following completion of successful treatment.

3. Three amebic colitis patients developed typical chronic idiopathic ulcerative colitis (Bargen's type I) less than two months after cure of amebiasis and after the return of their sigmoidoscopic pictures to essential normality. It is probable that there was no causal relationship.

4. The patient with multiple amebomas of the transverse colon was studied for 23 months. The static radiologic nature of the tumors over the 20 month period prior to medical eradication of the infection, and their complete disappearance following cure were notable.

5. The treatment schedules and results are given in detail. Two patients (5 per cent of the series) spontaneously lost their infections without treatment. The other 38 required a total of 62 courses of treatment for cure, as determined by rigid criteria. One case of amebic hepatitis was cured without emetine, and two of liver abscess without surgical procedures.

6. There was no constant or predictable relationship between the size of drug dose and its effectiveness in amebic colitis.

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# THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

## III. TECHNIQS FOR THE RECOVERY OF THE EGGS

### OF SCHISTOSOMA JAPONICUM

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#### INTRODUCTION

In an earlier communication in this series of studies (1) it has been indicated that positive confirmation of presumptive clinical diagnosis of schistosomiasis japonica depends on demonstration of the egg of the etiologic agent, *Schistosoma japonicum*. In the average individual in a heavily endemic area direct fecal film examination usually reveals one or more eggs of the parasite for each cover-glass preparation. On the other hand, lightly infected persons, or those who have submitted to one or more courses of antimony treatment but are not cured, frequently have so few eggs in their stools that repeated examination of the feces without concentration technics fails to provide confirmatory evidence. For this reason methods for the concentration of eggs from larger amounts of feces are required. Faust and Meleney (2) utilized sedimentation and also a hatching technic, the advantages of which were later demonstrated by Andrews (3). For the related species, *S. mansoni*, Faust and Hoffman (4) found one-half per cent glycerin in tapwater superior to plain tapwater for sedimentation. Recently Mathieson and Stoll (5) have found that hydrochloric acid-ether centrifugalization provides a higher yield of *S. japonicum* eggs than direct film, centrifugalization or zinc sulphate centrifugal floatation in tapwater, and Weller and Dammin (6) have demonstrated that the addition of a very small amount of certain detergents to hydrochloric acid greatly increases the number of *S. mansoni* eggs obtained per given mass of feces.

Because of the considerable number of military patients who have had clinical findings suggestive of schistosomiasis but with stools consistently negative for *S. japonicum* eggs by the usual technics, it was deemed advisable to make an intensive study of methods whereby greater confidence could be placed in stool examination in this disease. Moreover, it has been demonstrated by one of us (E. C. F.) (7), as well as by Lt. N. L. Hairston,<sup>4</sup> that there is a wide range in the types of immature, mature and degenerate eggs of this parasite which are evacuated in the stool, depending on the age of the infection, its relationship to chemotherapy and possibly other supervening conditions. In individuals passing so

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<sup>4</sup> Hairston, N. L.: Guide for the identification of the ova of *Schistosoma japonicum*. Office Chief Surgeon, AFPAC, 10 June, 1945.



few eggs in their stool that they are apt to be missed by direct fecal film examination, it is desirable to have a simple technic which will concentrate the eggs present in one to several grams of stool in a volume of processed material small enough to be examined in a short time. These eggs should be as free of fecal detritus as possible, in a good diagnosable condition and should be representative of all stages passed in the excreta. The technic should likewise provide for the recovery not only of typical mature eggs but also the immature stages and different types of degenerate ones.

#### MATERIAL AND TECHNIQS

*Material.*—The material which was utilized consisted of stools of patients infected with *S. japonicum*, before, during and subsequent to chemotherapy, and those of infected dogs.

*Technics.*—1. *Direct fecal film.* Minute amounts of formed, semi-formed or liquid feces, estimated to amount to about 10 to 20 mgm., were each suspended in about 5 to 10 parts of tapwater on a clean fecal slide measuring 75 x 37 mm. and mounted with a 22 mm. sq. cover glass. Ideally this was sufficiently clear to read newsprint through the film, but at times undigested starch granules in the feces made the preparation semi-opaque. One to eight (usually three to eight) films of this type were made from different parts of the stool. If flecks of bloody mucus were present, portions of these were also mounted for examination. All films were completely examined and all eggs of *S. japonicum* counted and their stages noted.

For comparative tests it was desirable to have a series of eight direct fecal films from the same positive stool in which at most 2 or 3 eggs were present on one or two films and the others were negative, as, for example, 1, 0, 1, 0, 0, 0, 0, 0; 1, 0, 1, 0, 0, 0, 1, 1, and 0, 0, 0, 0, 0, 0, 0, 0. On the other hand, when direct fecal film counts were high, as, for example, 152, 172, 131, the stool was at times very thoroughly mixed with that of uninfected human feces in the proportion by weight of 1 to 24, so as to reduce the egg count to a small number per film.

It was realized that these counts were not quantitative, since the minute amount of feces utilized was estimated rather than weighed. However, experience demonstrated that chance distribution of the eggs in the fecal mass was very irregular, so that no object would have been gained in more accurate determination of such small masses of material.

2. *Sedimentation.* The sedimentation glasses consisted of inverted cones of 300 ml. capacity provided with a lip and mounted on a base like a goblet. Five grams of stool (or, if liquid, 5 ml.) were removed from a specimen which had been thoroughly stirred up manually and were suspended in the liquid from which sedimentation was to take place. The following liquids were tested for their comparative sedimenting efficiencies:

- (1) Tapwater
- (2) 0.5% glycerin in tapwater
- (3) 0.1% Triton and 0.05% Triton in tapwater
- (4) 0.5% glycerin + 0.1% Triton in tapwater

- (5) N/10 KOH in tapwater
- (6) 0.5% glycerin + N/10 KOH in tapwater
- (7) 0.5% glycerin + 0.05 per cent Tween-80 in tapwater
- (8) 0.5% sodium sulphate in tapwater
- (9) 0.5% glycerin + 0.5% sodium sulphate in tapwater
- (10) N/10 KOH + 0.1% Triton in tapwater
- (11) 0.8 mg. % ferric ammonium alum in tapwater.

Tests were made to determine the usefulness of gauze in screening out the coarser elements in the feces. Dampened Curity surgical gauze was employed and comparative quantitative studies made on the efficiency of no gauze, one, two, three, four, five, six and seven layers. Measurements of the gauze employed indicated that it had a relatively constant mesh per square inch of 12 x 20 to 13 x 21 (based on eight separate measurements from a similar number of samples).

Numerous tests were made on the time which was required for sedimentation and the number of decantations advantageous to give a satisfactory sediment. Frequently counts were made on the eggs trapped in the gauze and in the water which was poured off. In the latter test each amount of supernatant fluid poured off was centrifugalized separately and total counts of eggs were made from each centrifugate.

By trial it was found that a very high egg sampling of the sediment was obtained by drawing off three one-tenth ml. amounts in a wide-mouthed pipette provided with a 0.1 ml. mark and fitted with a good quality rubber bulb, one amount from the top layer of sediment, one from the middle and one from the bottom. One-tenth ml. was utilized because it was usually just enough to be covered by and viewed clearly under a 22 mm. sq. coverglass. Frequently it was found desirable to count all eggs in the residual sediment after the three one-tenth ml. samples had been removed, to determine the percentage of the total eggs in the sediment which had been removed in the 0.3 ml. samplings.

In order to determine if 5 gm. samples of stool were adequate to counteract unequal distribution of the eggs in the specimen, two to four samples were frequently sedimented at one time, utilizing different parts of the manually mixed unprocessed specimen.

3. *Zinc Sulphate Centrifugal Floatation.* This was tested according to the original method (8), as well as following sedimentation.

4. *Ether Centrifugalization.* The basic method employed was a modification of the Telemann technic utilizing the proportions described by Mathieson and Stoll (5). One gm. of stool (or, if liquid, one ml.) was measured into an ordinary laboratory test-tube of about 35 to 50 ml. capacity. Five ml. of 40 per cent stock HCl (15 per cent conc. HCl) or substituted reagent were added and the feces thoroughly suspended in it. When two or more aqueous reagents were to be tested at the same time, they were mixed before being added to the feces. The suspension was next poured through two to four layers of dampened Curity gauze into a graduated 15 ml. centrifuge tube, an amount of ether equal to the suspension poured in, and the material carefully but thoroughly mixed. The suspension was then placed in an electric centrifuge and spun at 1500 or 2300

rpm for one or two minutes. When centrifugalization had been completed the tube was removed, the film at the interphase between the two strata was gently stirred in an attempt to produce settling of trapped eggs, and after one minute had been allowed for settling, all of the supernatant fluids were decanted. The sediment was then agitated by tapping the tube vigorously with the middle finger, was poured onto fecal slides, mounted with coverglasses and the eggs counted.

The following reagents were employed in the ether centrifugalization technic:

- (1) 5 ml. 15% HCl (sp. gr., 1.08) (Mathieson and Stoll) (5)
- (2) 5 ml. 15% HCl + 0.06 ml. Triton (Weller and Dammin) (6)
- (3) 5 ml. 15% HCl + 0.06 ml. Tween-80
- (4) 5 ml. 15% HCl + 0.06 ml. Triton + 0.25 ml. formaldehyde
- (5) 5 ml. 9.75% KOH (mol. wt. equivalent of 15% HCl)
- (6) 5 ml. 9.75% KOH + 0.06 ml. Triton
- (7) 5 ml. 9.75% KOH + 0.06 ml. Tween-80
- (8) 2.5 ml. 15% HCl + 2.5 ml.  $\text{Na}_2\text{SO}_4$  (sp. gr., 1.08)  
(sp. gr. of  $\text{Na}_2\text{SO}_4$  was used because of the difficulty in obtaining an accurate solution of this salt due to its varying water content)
- (9) 2.5 ml. 15% HCl + 2.5 ml.  $\text{Na}_2\text{SO}_4$  (sp. gr., 1.08) + 0.06 ml. Triton
- (10) 3.0 ml. 15% HCl + 2.0 ml. 33.5%  $\text{ZnSO}_4$  + 0.06 ml. Triton
- (11) 5 ml.  $\text{Na}_2\text{SO}_4$  (sp. gr., 1.08)
- (12) 5 ml.  $\text{Na}_2\text{SO}_4$  (sp. gr., 1.08) + 0.06 ml. Triton

Quantitative counts were made in every test on all the eggs in the sediment, and estimates were made from samples taken on the eggs trapped in the gauze and remaining in the supernatant liquids.

Careful examination was made at different stages in representatives of each modification of the ether centrifugalization technic to determine the diagnostic quality and the relative quantity of eggs of different types (i.e., immature, mature, degenerate, or those distorted by the technic) at each stage in the procedure.

Many quantitative counts were made to compare different ether centrifugalization technics with one another and with the direct fecal film and sedimentation methods.

5. *Hatching of miracidia.* Repeated hatching tests were made from sedimented stools and will be reported in another communication from the Commission on Schistosomiasis. They were not employed in the Commission's studies on diagnosis, since they provide evidence of the presence only of mature hatchable eggs and leave out of account the possibility of immature or degenerate types.

It has been stated above that comparative tests were made simultaneously on two or more technics or variations in technics in order to make a comparative evaluation of their efficiency. Direct fecal films were always made as a rough test of the number of eggs in samplings from different parts of the stool specimen. After a satisfactory sedimentation method had been developed, and tried out again and again to demonstrate its dependability, one or more sedimentations were always run as pilots on the zinc sulphate and ether centrifugalization technics. In general, as many comparative tests were conducted each day over a period of several weeks as could be handled by the three investigators concerned

with this special problem, while additional studies were conducted subsequently by two of the group (ECF and JWI), to check certain technical procedures and practical applications.

Before any series of studies was undertaken the three investigators checked one another to determine their individual ability to recognize the eggs of *S. japonicum* in all stages of development and degeneration, as well as accurate coverage of all of the material on a microscopic slide. The variation in count was usually about 5 per cent and was always less than 10 per cent. In quantitative studies shells from which hatching had occurred were counted and miracidia swimming in the water with sedimented material were not counted.

## PRESENTATION OF RESULTS

### *General Statement*

Results obtained in the efficiency of different concentration technics differed within rather wide margins, depending in part on the consistency and composition of the stool, in part on the organic material in the tapwater on a particular day and in part on the properties of a particular reagent or group of reagents to concentrate in a readily diagnosable state a high proportion of all eggs originally in the stool specimen utilized.

If the stool contained a considerable amount of mucus, either in microscopic masses or distributed through the feces, laborious stirring of the entire specimen never served to provide a completely homogeneous distribution of *S. japonicum* eggs. If the stool contained considerable vegetable roughage, it was necessary to screen this out through gauze. This separation was usually successful without loss of an appreciable percentage of eggs, but large flecks of mucus were also usually screened out and they frequently contained a higher percentage of eggs per volume than the feces. When the stool contained a large amount of minute starch granules, these were slow in settling and frequently held eggs in suspension, due to the property of maturing and mature viable eggs in causing a layer of fine particulate matter to stick to the outside of their shells. Moreover, if starch granules were sedimented with the eggs or were thrown down in the centrifugate, they clouded the microscopic field and partially obscured the identifying characters of the eggs.

On mornings when a high percentage of organic material was present in the tapwater difficulty was experienced in getting satisfactory sedimentation within a period of one to two hours, or it was practically impossible to determine whether the cloudy supernatant fluid contained eggs or only iron and organic material in finely particulate or colloidal suspension. Minute air bubbles in the water also from time to time retarded sedimentation. Distilled water was hard to obtain from the Medical Supply Depot and the Commission had no distillation apparatus of its own. Moreover, for practical purposes it seemed desirable to do the sedimenting with tapwater, since this, or equally turbid river and shallow well water, was all that was available to most of the hospital and medical laboratories in the endemic areas for use in fecal examination.

The property of reagents used in the ether centrifugalization technic to distort

or destroy some of the eggs was of critical importance, because it might provide a negative diagnosis when the sample of stool being processed might have contained a few eggs which should be recovered by a reliable concentration method.

The experimental tests will be presented in the order in which they were originally studied, namely (1) sedimentation, (2) zinc sulphate centrifugal floatation and (3) ether centrifugalization.

### *Sedimentation*

*Layers of gauze.* Many tests were made to determine the number of layers of gauze most effective in screening out large particulate material and at the same time to allow eggs of *S. japonicum* to pass through into the sedimentation glass. Although the tests varied somewhat, depending on whether tapwater alone was used or with some wetting or precipitating reagent, there was a definite trend which may be summarized as follows: (a) No screening provided little superiority over an average of five to eight direct fecal films; (b) There was added efficiency in egg concentration as the number of layers of gauze increased from one to four; (c) four to six layers provided approximately the same maximum yields, and (d) seven layers gave considerably diminished returns. Because of these findings and to simplify the procedure (Curity Absorbent Gauze is cut and folded in four layers) four layers were adopted as standard. Capt. A. V. Hunninen, Sn.C., parasitologist of the 118th General Hospital Laboratory on Leyte, had independently found four layers of surgical gauze to be very satisfactory.

*Sedimenting Medium.*—Tapwater was first tested without any wetting or precipitating reagents, since this was routinely employed in General Hospital laboratories carrying out routine diagnosis for *S. japonicum* eggs in the endemic area. Five-tenths per cent glycerin in tapwater was then utilized as a control because it had been found superior for recovery of the eggs of *S. mansoni* (4). Iron alum (as a substitute for potassium alum which was not available) was then tested as a precipitating reagent, using 0.8 mg. per cent in tapwater as employed by municipal water plants. N/10 potassium hydroxide and 0.5 per cent  $\text{Na}_2\text{SO}_4$  were employed in an attempt to digest mucus tags from around egg shells, alone in tapwater and in combination with 0.5 per cent glycerin. The detergents Triton NE and Tween-80 were studied because of their demonstrated wetting properties, in amounts of 0.1 and 0.05 per cent, in tapwater, with 0.5 per cent glycerin in tapwater and with N/10 KOH in tapwater.

In seventeen tests, in which two or three of these reagents or associated reagents were compared with one another for relative efficiency, the following results were consistently observed: (a) Sedimentation in tapwater gave an average concentration efficiency for *S. japonicum* eggs not in excess of 1.5 (i.e., about one-third of the fecal material had been removed), when checked against an average of five to eight random 22 mm. sq. cover-glass samplings of unprocessed feces. (b) Triton and Tween-80 proved to be good wetting agents but were consistently inferior to other reagents because they swelled the bulk of the sediment. For that reason, 0.1 per cent was less efficient than 0.05 per

cent, but even the latter was little better than tapwater without a reagent. (c) Iron alum always caused rapid and almost complete precipitation of particulate material in the suspension, hence none of the eggs were caught in the surface film or in the supernatant fluid after a period of a half-hour or more, and thus none were poured off. Hence, the total yield of eggs in the sediment was practically the total of eggs in the original sample of feces. However, iron alum swelled the bulk of sediment so that a given fraction at times yielded no more than tapwater sedimentation. When N/10 KOH was tested side-by-side with 0.5 per cent glycerin and with 0.5 per cent glycerin + N/10 KOH, the N/10 KOH alone yielded only 15 per cent of the number of eggs recovered by 0.5 per cent glycerin, while the combination of the two reagents gave only one-half the yield of glycerin alone. Sodium sulphate was very satisfactory in dissolving mucus and cleaning the surface of egg shells but caused approximately a 20 per cent swelling in the bulk of the sediment. In association with glycerin it neither increased nor decreased the egg yield, although it added to the clearness of outline of the eggs and therefore simplified identification.

From its earliest application 0.5 per cent glycerin in tapwater proved to be very successful in providing an egg yield which was consistently good, usually yielding three- to twenty-fold or more concentration per 22 mm. square cover-glass compared with an average of direct fecal films. The eggs of all stages were well preserved and the mature viable ones, on being washed in filtered river water with a pH of 7.6, readily hatched.

*Relation of sampling to total number of eggs in sediment.*—As indicated above under "Material and Technics," it was decided to take samplings of the sediment just sufficient to be covered conveniently by a 22 mm. square cover-glass. This amounted to 0.1 ml. To obtain an average sampling of the entire sediment, one 0.1 ml. amount was drawn up from the top layer of sediment, another from the middle and a third from the bottom.

In repeated tests (utilizing both human and dog's stools) there was no consistency in highest egg concentration from the different strata. The egg yields from three 0.1 ml. samplings of sediment in nine representative tests are summarized in table 1.

*Relation of egg yield to number of decantations.*—Although a very turbid suspension in tapwater or 0.5 per cent glycerin in tapwater, with or without 0.05 per cent Triton, might require five to seven decantations for the supernatant fluid to become perfectly clear, an attempt was made to reduce the decantations to three. With glycerinated water as the sedimenting medium, counts were made on three 0.1 ml. amounts of sediment following one, two and three decantations. The number of eggs recovered from the 0.3 ml. sampling compared with the combined three 0.1 ml. samplings plus residue indicated that the 0.3 ml. amount after one decantation yielded 21.5 per cent of all the eggs, the 0.3 ml. amount after two decantations yielded 25.8 per cent and the 0.3 ml. amount after three decantations yielded 36.9 per cent of the total number of eggs in the container. Additional tests have demonstrated that more than three resuspensions and decantations do not justify the time employed.

*Time allowed for sedimentation.*—This depended on the type of stool which was being processed. Occasionally the turbidity of the supernatant fluid was not removed completely, even when an excess of Triton or of alum was added again and again. Usually, however, one to two hours was employed for the first settling, 30 to 45 minutes for the second and 30 minutes for the third and last sedimentation. Frequently within two and one-half to three hours after the sedimentation was begun it was practical to draw off material for microscopic examination, with the evidence that good concentration had been obtained.

*Eggs poured off in water of decantation.*—In order to gain some idea of the percentage of eggs in 5 gms. of the original unprocessed stool which were lost when the supernatant fluid was poured off the sediment, in certain tests each of the three volumes of water decanted was separately centrifugalized and the total centrifugate searched for eggs, for comparison with the three 0.1 ml. samplings

TABLE 1

*Egg yield from three 0.1 ml. samplings of sediment compared with total yield from sediment derived from 5 grams of stool and three direct fecal films*

(Utilizing dog's stool)

SEDIMENTATION TECHNIC	THREE 0.1 ML. SAMPLINGS	% OF TOTAL EGGS IN SEDIMENT	TOTAL EGGS IN SEDIMENT	EGGS TRAPPED IN GAUZE*	EGGS IN SURFACE FILM*	EGGS IN DIRECT FE- CAL FILMS	APPARENT CONCENTRATION
(1) 8 mg.% alum....	467	33.0	1415	2	0	133	3.5
(2) 0.5% glycerin....	1564	59.9	2611	0	0	250	6.26
(3) Idem.....	47	53.4	88	1	1	3	15.6
(4) Idem....	175	29.4	596	0	1 (shell)	9	19.4
(5) Idem.....	243	31.1	782	4	0	7	34.7
(6) Idem.....	41	42.7	96	0	0	2	20.5
(7) Idem.....	5	45.4	11	0	0	0	
(8) Idem.....	378	24.1	1570	19	0	7	54.0
(9) 0.5% glycerin + 0.5% Na <sub>2</sub> SO <sub>4</sub> ....	51	42.5	121	0	0	2	25.0

\* Sample tests only, not total counts.

and residue of original sediment. A representative experiment, in which 0.5 per cent glycerin in tapwater was employed, provided the following results: The centrifugate (0.6 ml.) from the first decantation contained 21 eggs; from the second decantation, (0.5 ml.), 12 eggs; from the third decantation (0.5 ml.), 33 eggs; the three 0.1 ml. samplings of original sediment, 691 eggs; residue sediment (2.5 ml.) 1327 eggs. Thus, only 3.2 per cent of all eggs in the original 5 gms. of feces was lost in the pour-off and 33.1 per cent of the total was recovered in 0.3 ml. of sedimented feces or in only 6.8 per cent of all sedimented and centrifugalized material (i.e., 2.8 ml. + 0.6 ml. + 0.5 ml. + 0.5 ml. = 4.4 ml.).

Another test was conducted similarly except that Triton (0.05 per cent) was added to the decanted water to secure more effective settling of fine particulate matter. Seven per cent of the total number of eggs in the original 5 gm. sample of stool was lost in the water of decantation and 23.4 per cent of the total was

recovered in the 0.3 ml. of original sediment sampled, which was 6.1 per cent of the total amount of original sediment plus that obtained from settling of the suspended material in the decanted waters.

*Comparison of egg yield in parallel sedimentation.*—From time to time, in order to serve as a gauge of the equal or unequal distribution of eggs in 5 gm. samplings of stools which had been homogenized as thoroughly as possible by manual manipulation, two or more sedimentation glasses were set up side-by-side. Representative tests are presented summarily in table 2.

*Summary of experiments on sedimentation.*—As a result of a long series of tests with yields of diagnosable *Schistosoma japonicum* eggs of all types by sedimentation technics, in which various wetting and precipitating reagents were tried, a simple efficient method was developed for the recovery of a relatively high percentage of the total eggs from 5 gms. of original unprocessed

TABLE 2

*Comparative egg yields (first three 0.1 ml. of sediment) in parallel sedimentations using 0.5 per cent glycerin in tapwater*  
(Utilizing dog's stool)

GLASS	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	REMARKS
Series 1	47	30				Feces with mucus
Series 2	146	167				Semi-formed feces
Series 3	31	16				Formed feces
Series 4	52	51				Semi-formed feces
Series 5	175	143				Semi-formed, homogenous
Series 6	6	4	4	1	5	Well-formed feces
Series 7	3	1	1			Well-formed feces

The results indicate that approximately equivalent yields can not be predicted on the basis of the consistency of the stool. In general, however, a semi-formed stool seems to provide a more homogeneous distribution of the eggs than a formed one or one with macroscopic mucus.

stool in three 0.1 ml. of sediment, or three 22 mm. square coverglass preparations. The original stool was thoroughly suspended in 250 ml. of 0.5 per cent glycerin in tapwater, strained through four layers of Curity surgical gauze, and allowed to sediment for one to two hours. The supernatant water was then carefully decanted, the sediment resuspended in 250 ml. of 0.5 per cent glycerinated water and again allowed to sediment for about 30 to 45 minutes. After a second decantation, resuspension of the sediment in 250 ml. of 0.5 per cent glycerinated water and settling of the suspension a third time for about 30 minutes, the supernatant water was poured off and three 0.1 ml. of sediment (one from the top, one from the middle and one from the bottom stratum) were drawn off in a wide-mouthed pipette provided with a tightly fitting rubber bulb of about 2 to 3 ml. capacity. From 23 to 60 per cent of all eggs in the original 5 gms. of stool was recovered from the three coverglass preparations. If there was macroscopic evidence of mucus in the stool, the addition of 0.5 per cent sodium sulphate



to the original 0.5 per cent glycerin in tapwater reduced the likelihood of eggs being trapped in mucus flecks, hence being screened out in the gauze. Precipitating reagents, as alum, or wetting agents, as Triton, were not found of practical value, since without them only a relatively small number of eggs (3 to 7 per cent in quantitative tests) were poured off in the decanted waters, while the addition of these reagents swelled the bulk of the sediment and thus diluted the egg yield per volume of sediment.

### *Zinc Sulphate Centrifugal Floatation*

Experiments were set up to test the possible diagnostic usefulness of solutions of  $\text{ZnSO}_4$  as a reagent to separate eggs of *S. japonicum* by floating them out of a suspension of feces. Both 33½ per cent and 40 per cent solutions in distilled water were used. One gm. of feces was thoroughly suspended in about 15 ml. of the  $\text{ZnSO}_4$  solution and then centrifugalized at about 2300 rpm for one minute. One-tenth ml. of material each from surface film and from bottom sediment was

TABLE 3

*Schistosoma japonicum* egg yields following centrifugalization of sedimented stools in  $\text{ZnSO}_4$  solutions  
(Utilizing dog's stool)

0.1 ML. SAMPLE REMOVED FROM	PREVIOUS SEDIMENTATION IN TAPWATER		PREVIOUS SEDIMENTATION IN 0.5% GLYCERIN IN TAPWATER		PREVIOUS SEDIMENTATION IN 8 MG. % ALUM IN TAPWATER	
	33½% $\text{ZnSO}_4$	40% $\text{ZnSO}_4$	33½% $\text{ZnSO}_4$	40% $\text{ZnSO}_4$	33½% $\text{ZnSO}_4$	40% $\text{ZnSO}_4$
Surface film.....	0	18	23	30	14	13
Bottom sediment.....	74	64	169	53	43	47

*Note:* Direct fecal films (22 mm. square coverglasses) averaged 71 eggs. Sedimentation in tapwater yielded 171 eggs; in 0.5 per cent glycerin in tapwater, 254 eggs; in 8 mg. per cent alum 263 eggs. Each of these egg yields was obtained from 0.1 ml. of bottom sediment.

then removed and examined. Results are shown in table 3. Only a few distorted eggs were occasionally recovered from the surface film, while those in the sediment were distorted and were no more concentrated than in direct fecal films.

Attempts were then made to float eggs out of sediment obtained from 3 tapwater suspensions and decantations, similar treatment from 0.5 per cent glycerin in tapwater and from 8 mg. per cent alum in tapwater. Both 33½ and 40 per cent  $\text{ZnSO}_4$  were tested. The technic of centrifugalization was similar to that described in the paragraph immediately above, except that one ml. of sediment rather than unprocessed feces was utilized. The results are recorded in table 3.

Inspection of table 3 indicates the poor yield of eggs in the surface film and even the relatively mediocre yield in the bottom sediment following the zinc sulphate technic. While in some instances 40 per cent  $\text{ZnSO}_4$  solution was superior to 33½ per cent solution, this was not usual. In all instances the eggs were so distorted that diagnostic recognition was endangered.

These experiments were repeated twice, both with original stool and sediment, with similar results. The technic was thereafter abandoned as not suitable for use in the diagnosis of *S. japonicum* eggs.

Two series of tests were conducted with sodium sulphate solution having a specific gravity of 1.18 in an attempt to obtain satisfactory centrifugal floatation. Eggs remained in the bottom sediment and were badly shrunken. Finally formalin-fixed  $\text{ZnSO}_4$  and  $\text{Na}_2\text{SO}_4$  centrifugal floatation tests were carried out. In this series the surface film gave negligible results and the bottom sediment never equalled that of direct glycerin sedimentation.

### *Ether Centrifugalization*

Before ether concentration technics were investigated, sedimentation with 0.5 per cent glycerin in tapwater had been standardized as a dependable concentration method, so that direct fecal film counts and sedimentation counts were always made as controls of all ether centrifugalizations. Likewise, sample counts were always made on the number of eggs trapped in the gauze and those which remained in the interphase film between the ether and the heavier reagent (or reagents). In the beginning of this study quantitative counts were also made to determine the optimum number of Curity gauze layers required for maximum egg yield, beginning with no gauze screening and increasing to four. The results indicated that two layers rather consistently provided the highest egg harvest in the centrifugate.

The electric centrifuge available in the Philippines was a laboratory angle-arm model for use with 110 volts alternating current. At the time no opportunity was afforded for calibrating its speeds, so that estimates of 1500 and 2300 rpm were made on the basis of previous experience with this model. Following return of the senior investigator to New Orleans calibration of the same model was made and the speed estimates were verified. In addition, several tests were made on fecal samples containing *S. japonicum* eggs in comparing the results obtained with the angle-arm and the International clinical centrifuge at these two speeds. The almost identical enrichments obtained indicated that these two types of centrifuges may be employed with the relative certainty that the technic will be carried out equally well.

*Hydrochloric acid-ether technic.*—This was set up in an attempt to duplicate the work of Mathieson and Stoll (5) on *S. japonicum* stools, the technic of which had been published by Mackie, Hunter and Worth (8). The test was carried out several times. In each instance all of the centrifugalized sediment was examined microscopically and all of the eggs present in the films were counted. The results are presented in table 4. The evidence was convincing that this technic was superior to direct fecal films but was never better than, and usually inferior to sedimentation in 0.5 per cent glycerin in tapwater.

*HCl + Triton-ether technic.*—Because of the inferiority of HCl-ether centrifugalization to glycerin-tapwater sedimentation in the above-described tests and because unpublished information of the success of Weller and Dammin (6) who used the detergent Triton NE with HCl and ether in concentrating *S. mansoni*

eggs from feces, comparative tests with and without this reagent were undertaken. In series no. 3 of table 4, in which the HCl-ether centrifugate contained no eggs, the parallel tests with HCl + Triton-ether provided 25 eggs in one tube and 41 in another. This led to an inquiry into the reason for the remarkable difference in yields.

Observations had previously been made that sedimented material contained not only mature viable eggs of *S. japonicum* but an appreciable proportion of immature and degenerate ones, including occasional calcified eggs. Only mature eggs were recovered in the HCl-ether treated feces, while in the HCl + Triton-ether centrifugate all stages were obtained except calcified specimens.

A one-gm. sample of human feces containing many *S. japonicum* eggs of all types, as determined by direct fecal film examination, was first treated with 5 ml. of 40 per cent stock HCl (15 per cent conc. HCl) and the reagent thoroughly

TABLE 4

*Comparative results of direct fecal film, three 0.1 ml. of 0.5% glycerin-sedimented feces and HCl-ether centrifugate*  
(Utilizing dog's stool)

SERIES NO.	DIRECT FECAL FILM	GLYCERIN SEDIMENTATION		HCl-ETHER TECHNIC		
		Gauze sample	3 × 0.1 ml.	Gauze sample	Interphase film	Total centrifugate
1	17, 10, 6, 12, 23, 21, 18; av. 15	4	66 + 73 + 92 = 231	(1) 9 (2) 12	72 97	201 44
2	2, 0, 1, 0, 2, 1, 2, 1; av. 1.1	(1) 0 (2) 2	9 + 12 + 18 = 39 6 + 5 + 8 = 19	(1) 0 (2) 0	0 1	7 9
3	0, 1, 0, 3, 0, 0, 0, 2; av. 0.75	(1) 0 (2) 0	16 + 15 + 16 = 47 13 + 7 + 10 = 30	1	2	0
4	2, 2, 1, 1, 0, 1, 0, 0; av. 0.87	0	35 + 17 + 16 = 68	0	0	10

stirred into the feces to make a homogeneous mixture. Examination under the microscope showed good fixation of the eggs but no apparent loss or distortion of any except the calcified ones. Then 5 ml. of ether were added, gently stirred in and microscopic examination made of a small sample. No change had occurred. Next the mixture was vigorously shaken and a film of the shaken mixture immediately examined. Within a period of ten seconds some eggs, particularly the immature and degenerate ones, were becoming decorticated and the contents vacuolated, while others, including mature ones, were becoming swollen, with ballooned shells which "popped" during the short period of observation. A comparative test showed that with the addition of the Triton (0.6 ml. of 10 per cent solution) to the HCl before mixture with the feces this distortion and destruction of eggs was materially reduced. Thereafter HCl-ether alone was never employed.

Comparative studies were made between the efficiency of Triton and Tween-80 (another detergent available for trial). Likewise KOH (9.75 per cent, equivalent mol. wt. of 40 per cent HCl) was tested alone, with Triton and with Tween-80. The results of lengthy tests in which these four reagent combinations were compared are presented in table 5.

TABLE 5  
*Comparative efficiencies of HCl and KOH with Triton and Tween-80 in recovery of S. japonicum eggs*  
(Utilizing dog's stool)

SERIES NO.	DIRECT FILM	SEDIMENT (0.3 ML)	HCl + TRITON-ETHER	HCl + TWEEN-80-ETHER	KOH + TRITON-ETHER	KOH+TWEEN-80-ETHER
1	63, 74, 67; av. 68	1026	282		2693	
2	1, 0, 0, 0, 1, 0, 0, 0; av. 0.25	78	45	21	9	0
3	0, 1, 3, 0, 0, 2, 2, 2; av. 1.25	38	11	27	42	8
4	1, 3, 1, 1, 1, 0, 1, 0; av. 1.0	83	100	100	10	100
5	6, 6, 4, 0, 5, 4, 3, 5; av. 4.1	127	246	63	5	22
6	0, 0, 1; av. 0.33	3	7	3	2	2
7	3, 9, 4; av. 5.3	126	165			
8	0, 0, 0	1	1	1	2	1
9	0, 0, 0	1	0	0	0	1
10	8, 9, 3; av. 7	146	86	53	148	44
11	3, 1, 0, 0, 1, 0, 0, 0; av. 0.63	31	49	15	74	
12	1, 0, 1, 0, 0, 0, 0, 0; av. 0.25	52	13	27	21	
13	2, 1, 5, 2, 2, 3, 4, 5; av. 3	175	81	213	3	

The results of the tests summarized in table 5 indicate that direct sedimentation in 0.5 per cent glycerinated water and the ether centrifugalization technics almost invariably but not always provide egg yields many-fold greater than direct fecal films. While at times the ether technics tested give a better count than sedimentation, no single ether technic showed consistent superiority.

HCl + Triton-ether excelled in five of the thirteen tests; HCl + Tween-80-ether was superior in two; KOH + Triton-ether, was best in three and equivalent (148 vs. 146) in one, and KOH + Tween-80-ether, in only one. Had there been four sets of sedimentation tests set up in each series it seems possible that the advantage would have been consistently in favor of sedimentation. In series 8 and 9 the concentrates provided diagnosable eggs when the direct films were negative.

The inequalities and inconsistencies in the four ether concentration technics are not necessarily due to inherent faults in the technics. It seems more probable that they are the outcome of differences in the distribution of the eggs in the stools. Thus, the unusually heavy yield for KOH + Triton-ether in series no. 1 compared with HCl + Triton-ether may possibly have resulted from this inequality in distribution. In view of the demonstrated unequal distribution of eggs in 5-gm. samples used in sedimentation the chance of this inequality in one-gm. samples is much greater.

*Additional studies on distortion and destruction of eggs by reagents employed in ether centrifugalization.*—As the tests progressed there was some evidence that some egg loss occurred through destruction when HCl + Triton-ether technic was employed. This possibility was therefore investigated.

One ml. of a semi-liquid human stool containing many *S. japonicum* eggs was suspended in 5 ml. HCl + 0.06 ml. Triton and allowed to settle. Before screening through gauze 0.2 ml. of the sediment was drawn off and all eggs found were studied for any changes which might have resulted from contact with the reagents. There were 10 immature, 46 mature and 8 degenerate eggs, all of which were similar to eggs previously seen in direct films.

After screening, 5 ml. of ether were added, the mixture was gently agitated and after 30 seconds 0.2 ml. of sediment was removed for examination. Of the eggs found the majority were mature, including about 20 per cent with thinned, swollen shells. A few degenerate eggs were in the process of decortication.

Next the residue was thoroughly shaken and centrifugalized at 1500 rpm for one minute. Both total sediment and interphase film were examined. The sediment contained approximately 2000 eggs, 90 per cent of which were readily diagnosable. The remainder were badly distorted and some were ballooned. Only one partly decorticated degenerate egg was found. Several immature eggs were discovered, all of which became distorted, or popped open and disintegrated as observation continued. The interphase film contained 13 diagnosable immature and mature eggs, together with 17 immature and degenerate ones which were diagnostic risks. Paralleling this progressive test of the action of the reagents on the eggs, one ml. of the same stool was routinely processed by the HCl + Triton-ether method. The results were as follows: Gauze sample, 71 eggs of different types and stages of disintegration; interphase film (complete count), 4 immature, badly digested eggs, 3 mature, partly digested or disintegrating ones which were diagnosable, one empty shell and no degenerate ones; sediment, 2893 eggs, most of which were readily diagnosable.

This study indicated that, while Triton quantitatively improved the egg yield

as compared with HCl-ether alone, a considerable number of eggs were being distorted or destroyed. Thus, destruction of some of the eggs was giving a lower egg count than the actual one, while distortion of an appreciable number was endangering accurate quantitative counts. Immature, and particularly degenerate eggs were almost always distorted or destroyed. In a microscopic check of eggs in stools following chemotherapy, degenerate and immature eggs would almost certainly be destroyed, providing a possible false negative finding.

In order to determine the effect of other reagents supplementing or replacing HCl in the ether centrifugalization technic several new series of tests were undertaken. Formalin fixation of the eggs was attempted by adding 0.25 ml. of 40 per cent (stock) formaldehyde to 5 ml. of 40 per cent HCl, and similarly to 5 ml. of 40 per cent HCl + 0.06 ml. concentrated Triton. Qualitatively the eggs were in a good state of preservation, with relatively few ballooned eggs and no badly distorted ones. However, degenerate eggs were lacking and the total count was comparatively inferior.

Potassium hydroxide (9.75 per cent solution) was next used as a substitute for hydrochloric acid and proved to be on the average as satisfactory as the latter when each was combined with a small amount of Triton or Tween-80 as a wetting agent. The comparative results have already been presented in table 5.

The extensive series of tests with HCl and KOH treatment of stool, each with Triton and Tween-80 as a wetting agent, indicated that an ideal ether centrifugalization method for concentrating eggs of *Schistosoma japonicum* had not been developed. It had previously been shown that, when HCl is combined with ether and the mixture is vigorously agitated, a considerable portion of the total eggs are destroyed or distorted beyond diagnosable quality. While Triton increased the total yield, probably due to its wetting action, and therefore prevented screening out of many eggs in the gauze, and possibly to some extent in reducing the action of HCl in its physical combination with ether during centrifugalization, some eggs, particularly immature ones, were known to have been distorted, some were seen to be undergoing disintegration and degenerate ones at times had completely disappeared. Moreover, calcified eggs were inevitably lost through the chemical action of HCl. KOH, in an aqueous solution having the gram molecular weight of the HCl solution employed, showed no evidence of qualitative loss, so that immature and degenerate eggs survived centrifugalization with ether, yet the total yield was not usually as good as glycerin sedimentation.  $\text{Na}_2\text{SO}_4$  was next employed as a supplement to HCl.

*Na<sub>2</sub>SO<sub>4</sub> as a reagent in ether centrifugalization.*—As a result of extensive experience sodium sulphate is known to be a solvent for intestinal mucus. It constitutes a very satisfactory pre-treatment purgative before the administration of an anthelmintic for the elimination of hookworms or tapeworms which have their heads embedded in mucus of the small bowel. Five per cent solution of the salt serves as an excellent solvent for the mucous-like secretion of *S. japonicum* eggs, which accumulates on the outside of the shell and causes an agglomeration of cellular debris (7). It was therefore believed that it had properties which deserved careful study.

Because of the differences in water content of sodium sulphate U.S.P., even when in a previously unopened, tightly stoppered bottle, it was found necessary to make up a solution in water on the basis of specific gravity rather than grams per liter. Empirically this was tried in the same concentration as 40 per cent stock solution of HCl, since it was to be used as a partial replacement of this reagent in the ether centrifugalization technic. This provided a specific gravity of 1.08.

A preliminary series of experiments, in which  $\text{Na}_2\text{SO}_4$  was included, was carried out on a freshly passed stool of a patient who was discharging relatively few eggs of *S. japonicum*, many of which were degenerate. Direct fecal films (total 8) averaged 2 eggs per film. Glycerinated water sedimentation on 5 gms. of stool gave an egg yield of 378 for three 0.1 ml. of sediment, or 24 per cent of the total number of eggs in the entire sediment (3.5 ml.).

The ether technics utilized for comparison were as follows: KOH + Triton-ether; HCl + Triton-ether; HCl (2.5 ml. of 40 per cent conc.) +  $\text{Na}_2\text{SO}_4$  (2.5 ml. of sp. gr. 1.08) + Triton (0.06 ml. of conc. sol.)-ether, and HCl (3.0 ml. of 40 per cent conc.) +  $\text{ZnSO}_4$  (2 ml. of 33.5 per cent sol.) + Triton (0.06 ml. of conc. sol.)-ether. Although another member of the Commission, Major George W. Hunter III, Sn.C., had undertaken preliminary work with HCl,  $\text{Na}_2\text{SO}_4$  and Triton in ether centrifugalization before he joined the Commission (9, 10), neither his reagents nor technic were known to the senior investigator until after this series of tests had been initiated.  $\text{ZnSO}_4$  was tested at the suggestion of Lt. Col. Harry J. Bennett, who was on temporary duty assigned to the Commission.

KOH + Triton-ether yielded 28 eggs in the centrifugate and 37 eggs in the interphase film; HCl + Triton-ether, 198 eggs and 3 eggs respectively; HCl +  $\text{Na}_2\text{SO}_4$  + Triton-ether, 422 eggs and 0 eggs respectively, and HCl +  $\text{ZnSO}_4$  + Triton-ether, 338 eggs and 7 eggs respectively. Both of the latter two technics provided eggs of excellent diagnosable quality and on the basis of their superior yield promised much for further tests.

A second series of tests was conducted on a stool of the same patient three days later. Eight direct fecal films averaged 2.37 eggs; the first three 0.1 ml. of sediment from 5 gms. of stool yielded 243 eggs (31 per cent of total eggs in the sediment); KOH + Triton-ether, 178 eggs in centrifugate (with poor fixation); HCl + Triton-ether, 214 eggs (with fair fixation); HCl +  $\text{Na}_2\text{SO}_4$  + Triton-ether, 357 eggs (with good fixation), and HCl +  $\text{ZnSO}_4$  + Triton-ether, 153 eggs (with fair fixation). This demonstrated the possible superiority of HCl +  $\text{Na}_2\text{SO}_4$  + Triton over all of the other three combinations of reagents tested when both quality and quantity of the eggs were considered.

The following day a third series of tests was run on another patient's stool, with the following results. Eight direct fecal films averaged 0.5 egg; sedimentation (first three 0.1 ml.), 41 eggs (42 per cent of total eggs in sediment from 5 gms. of stool); KOH + Triton-ether, 29 eggs in the centrifugate; HCl + Triton-ether, 11 eggs; HCl +  $\text{Na}_2\text{SO}_4$  + Triton-ether, 33, and HCl +  $\text{ZnSO}_4$  + Triton-ether, 11 eggs. On the next day's stool from this patient 8 direct fecal films averaged 1.1 eggs; the first three 0.1 ml. of glycerinated water sedimentation yielded 55

eggs (38 per cent of total in sediment from 5 gms. of stool); KOH + Triton-ether, 0 eggs; HCl + Triton-ether, (a) 28 eggs, (b) 23 eggs; HCl + Na<sub>2</sub>SO<sub>4</sub> + Triton-ether, (a) 42 eggs, (b) 43 eggs, and HCl + ZnSO<sub>4</sub> + Triton-ether, 22 eggs.

On the basis of these findings it was concluded that Na<sub>2</sub>SO<sub>4</sub>, when added to HCl + Triton, provided a yield previously unachieved quantitatively and qualitatively in the ether centrifugalization method of concentrating *S. japonicum* eggs. Fewer eggs were trapped in the gauze, due to the action of sodium sulphate in setting eggs free from flecks of mucus and to the ability of this reagent in

TABLE 6

*Comparative yields of S. japonicum eggs with direct fecal film, sedimentation and ether centrifugalization, including sodium sulphate as reagent*

(Patients' stools utilized in tests nos. 1, 3, 5, 7, 8, 9; dogs' stools in tests nos. 2, 4, 6)

SERIAL NO.	8 DIRECT FECAL FILMS	SEDIMENTATION		ETHER CENTRIFUGALIZATION		
		3 X 0.1 ml.	% Total	HCl + Triton	HCl + Na <sub>2</sub> SO <sub>4</sub> + Triton	Na <sub>2</sub> SO <sub>4</sub> + Triton
1	Av. 2.37	32	34.3	40	59	82
2	Av. 0.87	21	34.4	22	62 29	12
3	Av. 0	3	23.1	2	2 3	5 4
4	Av. 39	691	34.2			1518
5	Av. 0	0		0		1
6	Av. 33.6	459	28.0			736
7	Av. 0			2	2	2
8	Av. 0	5	45.6	9	13	7
9	Av. 12	224	32.1		273 310	496 427

inhibiting the destructive physical action of the HCl-ether during centrifugalization. Attention was therefore turned to the possibility that sodium sulphate + Triton, without hydrochloric acid, might prove equally satisfactory and thus simplify the technic.

In order that the above-indicated study might be used for a comparative evaluation of the more promising technics which had already been tested, experiments were set up as previously, in which direct fecal films, glycerinated water sedimentation and three or four ether centrifugalization technics were run in combination. Both human and dog's stools were employed in these tests. The results of these tests are summarized in table 6.



From these series of tests the following conclusions were drawn. Sedimentation (with 0.5 per cent glycerin in tapwater) has a sustained yield several to many-fold that of direct fecal film. The HCl + Triton-ether technic is not particularly superior to this sedimentation. Wherever HCl + Triton was employed in series with HCl + Na<sub>2</sub>SO<sub>4</sub> + Triton, the latter was frequently superior, never inferior to the former. In the comparative tests of HCl + Na<sub>2</sub>SO<sub>4</sub> + Triton and Na<sub>2</sub>SO<sub>4</sub> + Triton, the latter produced higher yields five times, lower yields twice, and equivalent yield once. In series no. 5 Na<sub>2</sub>SO<sub>4</sub> + Triton provided the only positive diagnosis.

In conjunction with the above tests a careful qualitative study was made of the types of eggs found at successive stages of the three ether technics. This is presented in table 7.

In a later series of tests carried out by the senior investigator in New Orleans the number and percentage of eggs of different types of maturity and degeneration were obtained from studies on direct fecal films, glycerinated water sedimentation and different ether technics. These findings are recorded in table 8.

These data indicate that the increment in yield in the ether technics in which sodium sulphate is a reagent may be due in part to an added number of mature viable eggs but is primarily the result of the preservation in a diagnosable state of degenerate eggs. This is particularly important in chronic infections, in those undergoing antimony therapy where killed eggs are being evacuated and in cases where eggs may have undergone degenerative changes in putrefying stools before laboratory diagnosis has been undertaken.

In view of the apparent superior performance of ether technics in which Na<sub>2</sub>SO<sub>4</sub> either supplements or supplants HCl, and since the solution of Na<sub>2</sub>SO<sub>4</sub> originally employed was empirically on the basis of the same specific gravity as that of the HCl (5), (i.e., 1.08), it was deemed advisable to determine if this was optimum. Furthermore, it was important to find out if the speed of the centrifuge as employed (i.e., 1500 rpm) and the time of centrifugalization (i.e., 2 minutes) were optimal. The tests to study these factors were carried out by the senior investigator in New Orleans and are summarized in table 9.

The experiments presented in table 9 were somewhat limited because of scantiness of material in New Orleans at the time they were conducted. Those in Series I were carried out on one-gram specimens from a single stool and those in Series II on one-half-gram specimens from a single stool of a different subject.

Previous experience on Leyte, P. I. had conclusively demonstrated that centrifuge speeds less than 1500 rpm were not efficient in concentrating eggs of *S. japonicum*. Series II similarly indicates that 2300 rpm is not optimum. In speeds of less than 1500 rpm in angled or clinical centrifuges for 15 ml. tubes an appreciable number of eggs are not centrifugalized but remain in the supernatant fluid, particularly in the interphase film between the ether and aqueous solution. At 2300 rpm this was not the case: the loss in eggs was apparently due to their destruction, since they were not found in any portion of the tube's contents, while in II (c) those recovered were abnormal in appearance. With the centrifuges employed there were no intermediate speeds between 1500 and

2300 rpm. Thus, it may be tentatively concluded that 1500 rpm is approximately the most satisfactory speed for concentrating eggs of *S. japonicum*,

TABLE 7

*Progressive examination of S. japonicum eggs at successive stages in ether centrifugalization technics*  
(Utilizing dog's stool)

STAGE WHEN EXAMINED	(1) HCl + TRITON-ETHER	(2) HCl + Na <sub>2</sub> SO <sub>4</sub> + TRITON-ETHER	(3) Na <sub>2</sub> SO <sub>4</sub> + TRITON-ETHER
Stool suspension after shaking in reagent but before screening	Eggs well fixed but some suggestion of swelling and thinning of shell; no destruction or disintegration of eggs evident	Practically all immature and mature eggs less rotund than in (1); Degenerate eggs unmodified; all eggs more highly refractile and less blanched than in (1)	All type of eggs, including calcified ones normal in every respect; some trapped in mucus; many fatty globules in film
After screening through 2 layers of dampened gauze and gently shaking with ether	Most mature viable eggs more rotund than normal; several immature and degenerate ones with split shells; one degenerate egg shrunk; no progressive disintegration while viewed under microscope	No changes from normal	Eggs very highly refractile like bubbles in glass; normal in appearance under 4 mm. objective; few still trapped in mucus; some fatty globules in film
After centrifugalization: A. Interphase film	All eggs diagnosable but several split shells	No mature eggs seen; a few shrunk ones; no degenerate forms observed; one shell	Only 3 normal immature eggs seen; some mucus and oil globules
B. Centrigate	A few hundred rotund, readily diagnosable mature eggs; very few immature ones; no degenerate ones found	Hundreds of well preserved mature and nearly mature eggs; no early immature ones or degenerate ones found	Hundred of eggs in excellent diagnosable state; shell surface clean and smooth; internal structure of miracidia very nearly normal in appearance; appreciable number of degenerate and calcified eggs

treated with HCl + Na<sub>2</sub>SO<sub>4</sub> + Triton-ether or Na<sub>2</sub>SO<sub>4</sub> + Triton-ether and centrifugalized for 2 minutes in the usual type of clinical laboratory centrifuges.

TABLE 8

Number and percentage of *S. japonicum* eggs of different types observed in direct fecal film, glycerinated water sedimentation and different ether technics  
(Utilizing dog's stool)

TECHNIC	VIABLE IMMATURE	VIABLE MATURE	DEGENERATE	CALCIFIED	TOTAL
Direct Fecal Film (one 22 mm. sq.)	14 (15%)	35 (36%)	43 (45%)	4 (4%)	96 (100%)
Sedimentation (5 gms.)					
3 × 0.1 ml. sediment	20 (18%)	23 (20%)	63 (56%)	6 (5%)	112 (100%)
Total sediment	20 ( 5.6%)	113 (32.4%)	209 (59.8%)	7 (2.0%)	349 (100%)
Decanted water	0	2 (11.1%)	15 (83.3%)	1 (5.6%)	18 (100%)
Ether Technics (1 gm. each)					
HCl + Triton	12 ( 5.9%)	167 (82.3%)	21 (11.8%)	0	203 (100%)
HCl + Na <sub>2</sub> SO <sub>4</sub> + Triton	9 ( 2.3%)	313 (79.6%)	71 (18.0%)	0	393 (100%)
Na <sub>2</sub> SO <sub>4</sub> + Triton	14 ( 2.8%)	291 (59.9%)	166 (33.5%)	22 (4.4%)	496 (100%)

TABLE 9

Effects of different concentrations of sodium sulphate solution in ether technic, speed of centrifugalization and time of centrifugalization on the yield of diagnosable *S. japonicum* eggs  
(Utilizing dog's stool)

REAGENTS EMPLOYED	SP. GR. Na <sub>2</sub> SO <sub>4</sub>	RPM	CENTRIFUGE TIME	EGG YIELD				
				Im.	M-v.	Deg.	Calc.	Total
Series I								
HCl + Na <sub>2</sub> SO <sub>4</sub> + Triton	(a) 1.08	1500	2 min.	0	5	8	0	13
	(b) 1.08	1500	2 min.	0	11	5	0	16
	(c) 1.08	2300	2 min.	0	7	0	0	7
Na <sub>2</sub> SO <sub>4</sub> + Triton	(d) 1.08	1500	2 min.	0	7	10	0	17
	(e) 1.08	2300	2 min.	0	0	0	0	0
Series II								
HCl + Na <sub>2</sub> SO <sub>4</sub> + Triton	(a) 1.08	1500	2 min.	9	212	71	0	393
	(b) 1.08	1500	2 min.	6	374	142	0	522
	(c) 1.08	1500	1 min.	3	265	42	0	310
	(d) 1.06	1500	2 min.	5	200	68	0	273
	(e) 1.12	1500	2 min.	0	361*	121	3†	485
Na <sub>2</sub> SO <sub>4</sub> + Triton	(f) 1.08	1500	2 min.	14	294	166	22	496
	(g) 1.08	1500	2 min.	3	125	277	22	427
	(h) 1.08	1500	1 min.	10	105	187	13	315
	(i) 1.06	1500	2 min.	2	42	198	3	245
	(j) 1.12	1500	2 min.	2	113*	126	8	249
	(k) 1.06	1500	1 min.	6	144	98	11	259

Legend:—Im., immature; M-v., mature, viable; Deg., degenerate; Calc., calcified.

\* Many of these were dimpled on one side or partly collapsed.

† Single instance observed with HCl as reagent.

This series therefore indicates that the extensive experiments previously carried out with ether centrifugalization were within satisfactory limits of centrifuge speed.

In Series II there is evidence that one minute centrifugalization produces a lower egg concentration than 2 minutes. Moreover, at 2 minutes operation with 1500 rpm the number of eggs trapped in the interphase film or in the ether and aqueous solution above the centrifugate is very small when  $\text{HCl} + \text{Na}_2\text{SO}_4 + \text{Triton}$  or  $\text{Na}_2\text{SO}_4 + \text{Triton}$  are the reagents in which the particulate material in the positive stool is suspended. Before Triton and  $\text{Na}_2\text{SO}_4$  were employed the interphase film with  $\text{HCl}$ -ether had been conspicuous for the number of eggs which it trapped. (See table 4, above.)

#### DISCUSSION

Progress summaries have been provided in the presentation of the data in order that the reader might know why various modifications of basic technics were attempted and what results were obtained in comparison with those secured by the unmodified technics. Thus, the investigation developed into a step-by-step improvement of a few basic technics and the discarding of many tested modifications which were better than the originals but not equal to the best quantitatively, qualitatively or in reliability.

*Sedimentation.*—No detailed discussion is needed to interpret the usefulness of sedimentation in the recovery of *S. japonicum* eggs from feces. The extensive tests carried out in this investigation indicate conclusively that by simple procedures which may be carried out routinely in any clinical laboratory very considerable concentration of the eggs is effected. Of the many reagents employed glycerin in 0.5 per cent concentration in the water of suspension has proven to be consistently the best: (1) it is a good wetting agent; (2) it permits a very high percentage of the eggs in a stool specimen to sink to the bottom of the sedimentation glass, so that very few are poured off during decantation; (3) it does not swell the bulk of sediment as do detergents, sodium sulphate, alum or potassium hydroxide, so that a maximum number of eggs are present in a greatly reduced volume of finely particulate fecal material, and (4) it preserves the eggs in all stages of maturity or degeneration in the same state and proportion as they existed in the unprocessed stool.

A few remarks are appropriate with reference to the high yield of eggs in the first three 0.1 ml. samplings of sediment utilized as the test of concentration. At first these samplings were made entirely for comparison with the average direct fecal film. They provided so high a yield that it was deemed desirable to discover what percentage of the eggs in the total sediment was recovered in these samplings. This was obtained by the laborious process of counting the eggs in the entire residue of sediment after the first three 0.1 ml. had been withdrawn. The results were unexpected; namely, from more than 20 to as high as 67 per cent of the eggs in all of the sediment was pipetted out in the first three 0.3 ml. samplings, depending inversely on the volume of sediment from 5 gms. of stool (viz., 3.5 ml. to as little as 0.5 ml.). When the total sediment amounted to 1.5 ml. or more, several 22 mm. square coverglass preparations were required to exhaust

the residue. It was found that after approximately the first third of the residue had been removed for counting, the number of eggs per preparation was gradually reduced in subsequent films and in the last few preparations was at times zero. Moreover, in specimens from which no eggs were recovered in the first three 0.1 ml. samplings, no eggs were found in the residue sediment. The explanation may lie in the fact that the sediment was stirred up just as little as possible at the time each 0.1 ml. was withdrawn but there is also the suggestion that the eggs were sucked into the wide-mouthed pipette more rapidly than was the smaller (and usually lighter) fecal debris.

The specific gravity of *S. japonicum* eggs has not been determined, due to the fact that they shrink in the floatation medium (viz., zinc sulphate, sp. gr. 1.18 to 1.20) usually employed for protozoan cysts and the common helminth eggs found in feces, yet they remain in the bottom sediment.

If the stool specimen is well homogenized, the 5 gm. sample utilized for sedimentation in glycerinated water will prove to be adequate in almost every case for determining if the stool is positive or negative for *S. japonicum* eggs. Only when practically every egg in the stool specimen is in a mass of mucus is it possible that the technic may provide a false negative.

The one objection to sedimentation is that it is time-consuming. This is more than balanced by the seriousness of schistosomiasis japonica as a disease and the importance attached to the laboratory finding, since no method for specific diagnosis exists today other than demonstration of the egg of the parasite, either by stool examination or from proctoscopic material.

*Zinc sulphate centrifugal floatation.*—Because the eggs of *S. japonicum* shrink badly but fail to float in zinc sulphate solutions of 1.18 and 1.20 specific gravity, this technic is unsuited for concentration of these eggs. Even if they floated they would constitute a diagnostic risk, since they are so distorted that they are difficult to identify.

*Ether Centrifugalization.*—The modified Telemann HCl-ether centrifugalization technic, as employed by Mathieson and Stoll (5), has been demonstrated by the present investigators usually to provide superiority over direct fecal films but on the average to be considerably inferior to glycerinated water sedimentation. HCl digests all calcified eggs, in agitation with ether destroys immature and degenerate ones and at times destroys or distorts some of the mature viable ones. To some degree the addition of a small amount of the detergent Triton (0.06 ml. to 5 ml. of HCl) reduces this mechanical action and thus increases the egg yield but it lacks diagnostic safety in so far as immature and degenerate eggs are concerned. The one-half replacement of HCl with  $\text{Na}_2\text{SO}_4$  of the same specific gravity (viz., 2.5 ml: 2.5 ml.) in combination with 0.06 ml. of Triton NE increases both the quantity and diagnostic quality of the eggs and this combination of reagents may be regarded as very satisfactory from both viewpoints. Complete replacement of HCl with KOH, in combination with Triton NE, at times gave superior yield of eggs of relatively good diagnostic quality but the results were not sufficiently consistent to be recommended for routine work. The same applies to  $\text{ZnSO}_4$  solution in the ether centrifugalization technic. Similarly,

when the detergent Tween-80 was employed instead of Triton NE, the results were at times excellent, at other times only moderately good by comparison made on the same stool specimen. In the several series of tests, in which  $\text{Na}_2\text{SO}_4$  completely replaced HCl, the consistently best results were obtained. In the great majority of tests the quantity of eggs was greater than when HCl and  $\text{Na}_2\text{SO}_4$  were utilized half-and-half, due in part to a higher yield of mature viable eggs but to an even greater degree due to the preservation of all stages of immature and degenerate eggs, including calcified ones. The clinical importance of recovering these "atypical" eggs has been stressed previously (7) but can not be over-emphasized.

The changes in the ether centrifugalization technic from HCl to  $\text{Na}_2\text{SO}_4$  + Triton as the aqueous reagent before ether was added were developed by the present investigators only after prolonged tests, with intermediate and side steps to explore every likely possibility of improvement. Granted that completely quantitative data have not been obtained, nevertheless it may be stated with considerable certainty that marked improvement has resulted. In the first place, with  $\text{Na}_2\text{SO}_4$  + Triton no considerable number of eggs are strained out in the gauze. Secondly, eggs are not trapped in the interphase between the aqueous solution and the ether. Finally, eggs freed of mucus and detritus, of excellent diagnostic quality and in all stages, occur in the sediment in the same proportion as in the unprocessed stool.

The occasional failure of  $\text{Na}_2\text{SO}_4$  + Triton-ether to recover eggs when they are found by the sedimentation technic is probably not due to an inherent flaw in the technic. It will be remembered that eggs of *Schistosoma japonicum* are apt to be very unequally distributed in the stool specimen. For the ether technic only one gram of specimen is processed while five grams are employed in sedimentation. When two parallel tests were run with the same ether centrifugalization technic, there was frequently considerable variation in the egg yield. If five separate one-gram samples had been run in series, it is quite likely the egg yield from the total 5 gms. of stool would almost invariably have proven the superiority of the  $\text{Na}_2\text{SO}_4$  + Triton NE-ether to sedimentation. However, this would have increased to five-fold the number of films to be examined and in the long run would have been much more burdensome than sedimentation, in which only three cover-glass preparations are used for diagnosis.

Stools of both infected human beings and dogs were utilized in carrying out the various technics. During the earlier part of the investigation a constant source of human material was not available while at a later period both human and dog's stools were provided. The comparative study summarized in table 6 indicates that there was no difference in the behavior of the material from the two hosts. Moreover, careful observation has demonstrated that the types of eggs evacuated in the stools of recently infected human beings and dogs are similar in every respect, as are those in chronic infections of these two hosts. Thus, it seems justifiable to conclude that data obtained from infected stools of one of the hosts are, within the scope of this investigation, valid for the other host.

## CONCLUSIONS AND SUMMARY

1. *Direct fecal films.* These should always first be made and examined in all cases suspected of having schistosomiasis japonica. Three to eight such films provide considerable evidence of the presence of *Schistosoma japonicum* eggs in the stool and are particularly useful in indicating the unequal distribution of the eggs in a stool specimen. Moreover, direct fecal films are especially valuable for the examination of flecks of mucus, with or without blood, in which nests of eggs may be trapped.

2. *Sedimentation.* This technic is a simple diagnostic procedure whereby an appreciable amount of feces may be processed so that a relatively high concentration of *S. japonicum* eggs may be effected. For efficiency the specimen should be thoroughly homogenized manually, or preferably in an electric mixer. It should then be thoroughly suspended in at least twenty-five times as much 0.5 per cent glycerin in tapwater, then poured through gauze (or wire screening) to exclude macroscopic fecal debris. Four layers of Curity surgical gauze have been found to be very satisfactory for this purpose and serve to strain out only a very small percentage of the total number of *S. japonicum* eggs in the specimen.

A wetting agent aids the settling of the eggs. Dilute solutions of potassium hydroxide, iron alum, sodium sulphate and detergents all expedite sedimentation but they increase the bulk of the sediment and to that degree dilute the number of eggs per given volume of sediment. On the other hand, 0.5 per cent glycerin in tapwater does not swell the sediment and provides a yield of *S. japonicum* eggs in a completely natural state and in the same proportion as they occur in the unprocessed stool. Two and one-half to three hours after initiating sedimentation, following three suspensions and decantations, the final sediment may be sampled, with the assurance that a very high proportion of all the eggs will be concentrated in a small volume of sediment. Moreover, three one-tenth milliliter samplings taken from the sediment will deliver a high proportion (20 to 67 per cent) of all the eggs in the original unprocessed stool specimen.

3. *Zinc sulphate centrifugal floatation.* This technic is not satisfactory for recovery and diagnosis of *S. japonicum* eggs, since only a small percentage of the eggs float. Whether they float or remain in the sediment, they are shrunken and frequently constitute a diagnostic risk.

4. *Ether centrifugalization.* The tests may be conducted with either an International clinical laboratory centrifuge or an angle centrifuge, each carrying two or four 15 ml. tubes, which should be graduated. A weighed one-gram sample of stool, or if liquid one ml. sample, should be thoroughly homogenized in 5 ml. of the aqueous reagent to be employed and poured through two layers of Curity surgical gauze into the graduated centrifuge tube. An equal volume of ether should be added, the mixture manually shaken up and then centrifugalized at 1500 rpm for 2 minutes. The tube is removed from the centrifuge, the inter-phase film between the ether and the aqueous reagent carefully stirred with an applicator to permit settling of eggs caught in this layer, the contents allowed to settle for a minute and the entire supernatant liquid decanted. The small

amount of sediment is then transferred to one or more microscopic slides as required and examined for eggs.

Fifteen per cent hydrochloric acid-ether provides a sediment which usually although not invariably gives a higher egg yield than several direct fecal films. The mechanical interaction between the HCl and ether during centrifugalization destroys or distorts all immature and degenerate eggs, and at times some of the mature viable ones. The addition of certain detergents, particularly Triton NE (0.06 ml. per 5 ml. of HCl), increases the yield of eggs because it partially prevents their destruction. Hydrochloric acid (15 per cent) + sodium sulphate (sp. gr. 1.08) half and half + Triton is distinctly better than hydrochloric acid + Triton, both in egg yield and in diagnostic quality. Sodium sulphate + Triton (without hydrochloric acid) is usually superior, because it provides all stages of the eggs in the sediment in the same proportion as in the unprocessed stool and with excellent preservation. When other reagents, as KOH,  $\text{ZnSO}_4$ , CHOH and the detergent Tween-80, are added to HCl or substituted for HCl, they may at times produce excellent concentration and fair to good diagnostic quality of eggs in the sediment, but they are not as consistently reliable as is HCl +  $\text{Na}_2\text{SO}_4$  + Triton or  $\text{Na}_2\text{SO}_4$  + Triton.

5. For recovery of the largest number of *S. japonicum* eggs in a small amount (one gram) of stool, the  $\text{Na}_2\text{SO}_4$  + Triton-ether centrifugalization technic is the best one which has been developed. However, for routine stool examination, sedimentation utilizing 0.5 per cent glycerin in water is recommended, because the larger amount of stool processed, together with the high concentration of *S. japonicum* eggs in the first three 0.1 milliliter samplings withdrawn from the sediment, give somewhat greater assurance that, if eggs are present in the unprocessed stool, at least some will be recovered from this amount of the sediment. Both the  $\text{Na}_2\text{SO}_4$  + Triton NE-ether centrifugalization technic and sedimentation may be depended on to provide eggs of high diagnostic quality, including immature, mature, degenerate and calcified ones.

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# THE DEVELOPMENT OF THE VIRUS OF YELLOW FEVER IN HAEMAGOGUS MOSQUITOES<sup>1</sup>

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## I. INTRODUCTION

In a previous article (Bates and Roca-García, 1945a) we described the establishment of laboratory cycles of yellow fever virus using haemagogus mosquitoes and local monkeys. In these first experiments we observed that the infection of the mosquitoes seemed to depend on several factors, especially the amount of virus ingested by the mosquito and the environmental temperature at which the mosquitoes were maintained. More detailed studies of the factors governing virus establishment and development in these mosquitoes seemed to be warranted, and with this object in view continuous monkey-haemagogus transmission cycles, using a recently isolated virus strain, were maintained under laboratory control for a period of a year—fourteen complete mosquito-monkey cycles. A chart of these passages has been published in connection with a study of the behavior of the virus in the mammalian hosts (Bates and Roca-García 1946), and the serial position of any particular lot of infected mosquitoes can be determined by reference to this chart.

When we published our first paper on haemagogus transmission of yellow fever virus, we were still impressed by the technical difficulties of maintaining cyclic transmissions under laboratory control, and we concluded that special studies of virus behavior in mosquitoes would be most appropriately made by using as source animals monkeys inoculated with known amounts of virus. With more experience, we have reversed our opinion, and we now believe that a virus strain continuously maintained by "natural" cyclic passage provides the most satisfactory source of material for behavior experiments in either mosquitoes or mammals. The maintenance of a virus strain by this means cannot be undertaken as a casual or subordinate part of a laboratory program, but it is far from presenting insuperable difficulties if adequate facilities and personnel are dedicated to the project. It would probably be inadvisable to maintain more than one strain of a given virus by this means in a small laboratory because of the danger of possible confusion of material—always a very real hazard in virus experimentation. For this reason, our experiments have been largely made with a single strain of yellow fever virus, and it is thus impossible in some cases to be sure to what extent results are properties of yellow fever virus in general, and to what extent properties of the particular strain we used. The extent of variation among virus strains has been indicated in recent publications by work-

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ers in Brazil (e.g. Laemmert, 1944). In general, however, the various South American strains seem more similar to each other than any are to the African Asibi strain, which has been so extensively used in experimental studies of yellow fever; and we have observed nothing that would lead us to suspect that our Rodas strain was atypical.

As we have pointed out previously, the principal factors governing mosquito infection with virus seem to be (1) the characteristics of the virus strain, (2) the characteristics of the mosquito, (3) the virus dosage ingested, and (4) the environment of the mosquito, particularly the environmental temperature. We have tried to keep the virus strain factor constant, except for a few special experiments with possible modification through mouse brain passage. The mosquitoes used have in all cases been wild caught, and thus presumably represent a genetically diverse population. It seems very possible that a given wild population of haemagogus mosquitoes would include strains of varying virus susceptibility, but since we have not succeeded in establishing laboratory colonies of the Villavicencio species, it has been impossible to study this factor. The virus dosage factor is unquestionably of overwhelming importance in determining mosquito infection, but the lack of a precise method of quantitative estimation of the virus greatly complicates the study of this factor; this, and the effect of environmental temperature, have been the chief objects of our study.

The taxonomy of the mosquitoes of the genus *Haemagogus* is currently the subject of intensive study by several entomologists. The Villavicencio population, referred to in previous papers as *Haemagogus capricornii*, has been classified as a form of *Haemagogus spegazzinii* in a recent paper by Kumm, Osorno and Boshell (1946). The term "haemagogus" in publications from the Villavicencio laboratory may always be taken to refer to this specific population, since no other species of the genus seems to be represented in the region.

## II. MATERIALS AND METHODS

The materials and methods used in the present series of transmission experiments have been described in some detail in a previous article (Bates and Roca-García, 1945a). Mosquitoes for mouse inoculation were invariably killed with potassium cyanide, since Waddell (1945) has found that this method has no injurious effect on the virus content of the insects. We used a large "cyanide jar" made up according to the method long used by butterfly collectors: a few lumps of potassium cyanide are placed in the bottom of a jar and covered with a layer of two or three centimeters of sawdust; a layer of plaster of Paris is poured over this, and the plaster is allowed to dry and harden by leaving the jar open for 24 hours (somewhere outside of the laboratory!). A jar made up in this fashion will continue to give off cyanide fumes for a year or so, and the mosquitoes can be quickly and conveniently killed by placing the tubes that contain them inside of the jar; if the tubes are plugged with cotton, this need not be removed. Our infected haemagogus were always maintained in small tubes plugged with wire gauze, and these were very easily handled in the large cyanide jar. We also use this method for killing mosquitoes brought in from the field

for identification, making a jar large enough to hold our small field cages. The method is more convenient and cheaper than the use of chloroform.

We have not calculated precise titers and dosages in terms of mouse m.l.d. in the present article. The results of adult mouse inoculations with Rodas virus are very similar to those obtained with the Perez strain (Bates and Roca-García, 1945a), and the calculation of definite titers on such results would seem to us to be an example of the fallacy of misplaced concreteness. We believe that a better idea of the amount of virus of these strains in circulation can be obtained by giving the final serum dilution causing adult fatalities; this is meant to serve as an indication of the order of magnitude of the virus titer. We made a series of parallel inoculations in baby and adult mice, and then tested the surviving adult mice with "challenge" inoculations of fixed neurotropic virus according to the method of Fox (1943). We could thus compare mortality and immunity in adult mice with mortality in baby mice that had received

TABLE 1

*Parallel titration of Rodas virus in white mice of various age groups and in saimiri monkeys (serum of Saimiri 295, 4th day after infection by haemagogus of lot 284)*

DILUTION OF SERUM	MOUSE MORTALITY (INTRACEREBRAL INOCULATION OF VIRUS),* AGE OF MICE			CIRCULATION OF VIRUS IN MONKEY (INTRAMUSCULAR INOCULATION, 0.03 cc.)	
	7 days	21 days	44 days	Monkey no.	Result.
1:10			3/6		
1:10 <sup>2</sup>			4/6		
1:10 <sup>3</sup>		5/6	1/6		
1:10 <sup>4</sup>		5/6	3/6	310	Positive
1:10 <sup>5</sup>	3/4	3/6	0/6	331	Positive
1:10 <sup>6</sup>	2/4	2/6	0/6	343	Negative
1:10 <sup>7</sup>	1/4	0/6	0/6	345	Negative
1:10 <sup>8</sup>	0/4	1/6	0/5	347	Negative

\* Number dying over number inoculated.

identical inocula. The results were not consistent, in that adult mice frequently succumbed to challenge inoculations when the "baby" controls showed that the original inoculum had contained appreciable amounts of virus.

We have continued to find that "baby mice" (5 to 7 days old) give very regular results in titrations by intracerebral inoculation; and sound quantitative studies of virus behavior could be made using such animals. The results of a parallel titration in mice of various age groups and in saimiri monkeys were given in a previous article (Bates and Roca-García, 1945b, table 2), and the results of a similar but more complete titration are given in table 1 of the present article. We judge from this that 7-day-old mice on intracerebral inoculation are just as susceptible to virus of this strain as are saimiri monkeys on intramuscular inoculation; that the minimum lethal dose for 7-day-old mice corresponds very closely to the minimum infectious dose for saimiri monkeys. In the course of the experiments we have made many parallel titrations in baby

and adult mice. The end point with baby mice is very generally in the next tenfold dilution beyond the final dilution causing adult deaths; the titration reproduced in table 1 is unusual in this respect: we would have expected at least one adult death in the  $1:10^5$  dilution.

We also made a series of parallel tests for virus by inoculating 5- to 7-day-old mice intracerebrally and 3-day-old mice subcutaneously. The results of the subcutaneous inoculations were almost identical with those of the intracerebral inoculations. We judge from this that baby mice are sensitive indicators of virus transmission by mosquitoes, as reported by Bugher (1941). It would perhaps be more satisfactory to use the subcutaneous rather than the intracerebral inoculation of baby mice for tests of the presence of virus in mosquitoes, since intercurrent mortality from contamination or toxic materials in the inoculum would be greatly reduced.

### III. EFFECT OF TEMPERATURE ON VIRUS DEVELOPMENT

#### *Constant temperature: 30°C.*

The usual method of following the course of virus development in mosquitoes is by the inoculation of suspensions of pooled mosquitoes (Whitman, 1937). Since we found considerable variation in the virus content of individual mosquitoes, we decided to attempt to follow the history of the virus by the inoculation of suspensions of individual mosquitoes at regular intervals. The first such experiment was made with haemagogus of lot 181 which fed on Saimiri 176 on the 4th day after infection with rehydrated serum of Saimiri 99, the original animal of the Rodas series. The virus was thus 2 saimiri passages from man. The monkey circulated a considerable amount of virus, the serum on the 4th day killing 5/6 adult mice in  $1:10^4$  dilution (the highest dilution tested). The mosquitoes were kept at a constant temperature of 30°C. Five were inoculated in parallel groups of baby and adult mice immediately after feeding; 5 others were treated in the same way at 24-hour intervals through the first 10 days, and at 48-hour intervals through the next 10 days. Seventy-five mosquitoes were thus inoculated separately in mouse groups in the course of the experiment.

The mouse mortalities from these inoculations are given in detail in table 2, and the percentage of mice killed by mosquito inoculation on each day is plotted in graph form in figure 1. It will be noted that virus was recovered from all mosquitoes after the 3rd day except for 2 inoculated on the 14th day, i.e., from 53 of 55 mosquitoes (96 per cent). It seems hardly fair to include these 2 negatives in the mean for the 14th day, so this is based on the 3 positives only. Only 3 mosquitoes were killed on the 18th day, and 2 on the 20th day; these are averaged and the result plotted for the 19th day. Five mosquitoes bit baby mice on the 12th day, but none transmitted; 2 of 3 that bit on the 14th day transmitted. The "extrinsic incubation period" in this experiment can thus be taken as 13 days.

The curve for these mouse mortalities seems very similar to the curve that one

TABLE 2

Mouse mortalities caused by the inoculation of individual mosquitoes in parallel groups of adult (45 days) and baby (5-7 days) mice at regular intervals after the infectious meal (Haemagogus of lot 181 infected on Saimiri 176 and maintained at 30°C.)

DAYS AFTER IN- FECTIOUS MEAL	MOUSE MORTALITIES (A = ADULT MICE; B = BABIES)										TOTAL MORTAL- ITIES (IN PER CENT OF MICE)	
	A	B	A	B	A	B	A	B	A	B	Adults	Babies
0	2/5	5/5	3/6	4/4	4/6	5/5	3/5	5/5	3/6	4/4	50	100
1	0/6	1/5	2/5	5/5	3/6	3/5	1/6	5/5	0/3	0/5	23	56
2	1/5	2/5	0/6	0/0	0/4	0/0	0/6	1/5	0/6	2/5	4	33
3	1/6	5/5	1/6	5/5	0/6	0/5	4/5	5/5	0/6	3/5	20	64
4	0/5	3/5	2/5	3/5	1/5	3/5	1/6	5/5	2/6	4/4	22	75
5	3/5	5/5	2/5	5/5	2/5	5/5	2/5	5/5	0/6	4/5	36	96
6	0/6	1/4	1/6	5/5	5/6	4/4	0/6	2/5	2/6	5/5	24	74
7	0/6	4/5	0/6	1/5	1/6	3/5	0/6	5/5	1/6	5/5	7	72
8	2/6	5/5	2/6	5/5	2/6	5/5	2/6	5/5	2/5	5/5	38	100
9	3/6	5/5	3/6	5/5	4/6	5/5	1/6	5/5	4/6	5/5	50	100
10	3/6	3/3	2/6	4/4	6/6	5/5	1/6	4/5	2/6	5/5	47	96
12	4/6	5/5	4/6	5/5	2/6	5/5	1/5	5/5	3/6	5/5	48	100
14	3/6	4/4	0/4	0/4	2/6	4/4	4/5	5/5	0/6	0/5	(53)	(100)
16	4/6	5/5	3/6	4/4	3/6	5/5	3/6	4/5	6/6	5/5	63	96
18	5/6	5/5	4/6	5/5	6/6	4/4						
20	4/6	5/5	3/6	4/5							72	96

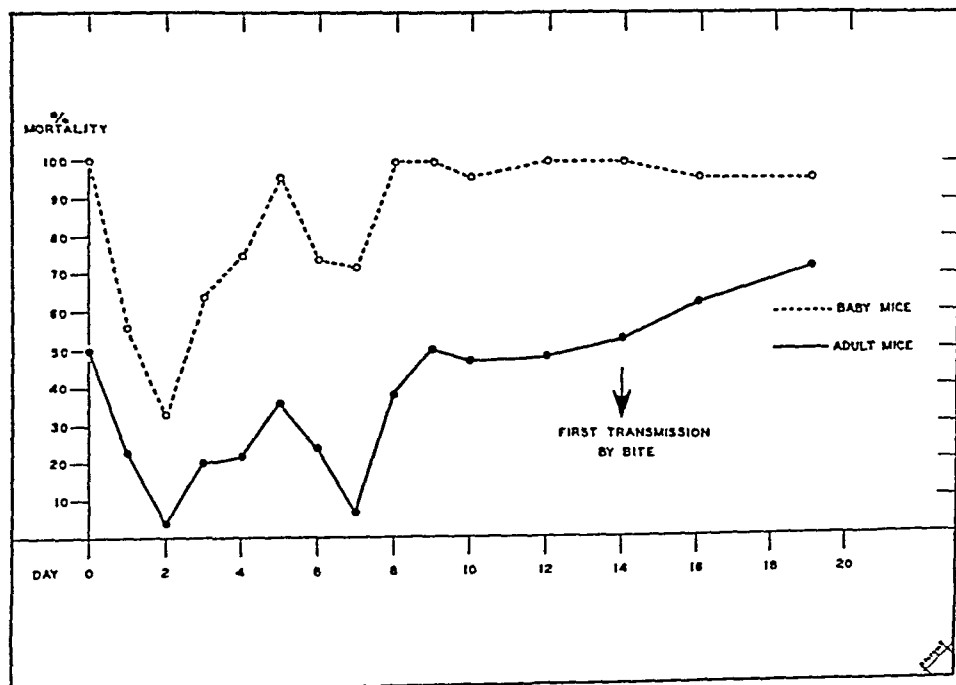


FIG. 1. MOUSE MORTALITIES FROM INOCULATION OF INDIVIDUAL MOSQUITOES. HAEMAGOGUS OF LOT 181, CONSTANT TEMPERATURE 30°C.

would expect for virus development in the mosquitoes, and it seems to be an index of the average amount of virus contained in the mosquitoes. The amount of virus measurable by this means has a definite limit: reached for baby mice on the 8th day, and by extrapolation for adult mice on about the 26th day. We have not been able to devise a satisfactory method of interpreting these mouse mortalities in terms of titer, even when the "average survival time" of the mice is taken into account: there are too many variable factors. Apparently there

TABLE 3

*Titration of pools of 5 mosquitoes each (Lot 234, infected on Aotus 9, kept at 30°C.)*

DAY	MOUSE MORTALITIES (5-TO-7-DAY-OLD MICE)				
	Pure	1:10	1:10 <sup>2</sup>	1:10 <sup>3</sup>	1:10 <sup>4</sup>
0	4/4	4/4	3/4	0/4	0/4
2	1/2	2/4	0/4		
4	4/4	1/4	0/4	0/4	
6	4/4	0/4	0/4	0/4	0/4
8	4/4	4/4	1/4	1/4	0/4
10	4/4	0/4	0/4	0/4	0/4
12	2/3	1/4	0/4	0/4	0/4
14	3/4	0/4	0/4	0/4	0/4
16	4/4	4/4	1/4	0/4	0/4
18	4/4	3/4	0/4	0/4	0/4
20	4/4	2/4	1/4	0/4	0/4
36	4/4	4/4	4/4	3/4	2/4

TABLE 4

*Titration of pools of 5 mosquitoes each (Lot 289, infected on Saimiri 342, kept at 30°C.)*

DAY	MOUSE MORTALITIES (5-TO-7-DAY-OLD MICE)					
	Pure	1:4	1:16	1:64	1:256	1:1024
0	4/4	4/4	4/4	3/4	1/4	1/4
2	0/4	0/4	0/4	0/4	0/4	
4	4/4	1/4	1/4	0/4	0/4	
10	4/4	1/4	0/4	0/4	0/4	
20	4/4	4/4	4/4	3/4	3/4	0/4

is a steady loss of virus during the first 48 hours after the infectious meal, but after 48 hours the virus has become established and starts to multiply. This multiplication is very slow, as compared with virus multiplication in the mammalian host. Transmission may simply represent a certain threshold of virus concentration in the mosquito, reached at 13 days in the case of this particular experiment.

We made several attempts to measure the rate of growth of virus in mosquito tissue by means of titrations of pools at regular intervals. To make the results comparable with those obtained from the inoculation of individual mosquitoes,

the pools were prepared by grinding 5 mosquitoes in 2.5 cc. of diluent (in our other experiments individual mosquitoes were ground in 0.5 cc. of diluent each) and this suspension was taken as the starting point in making serial dilutions. The results of 2 such titrations are given in accompanying tables: in table 3 those of lot 234, tested in tenfold dilutions; and in table 4 those of lot 289, tested in fourfold dilutions. The source animals for both of these experiments were circulating moderate amounts of virus: in the case of Aotus 9, the final serum dilution killing adult mice was  $1:10^5$  (3/6 mortality); in the case of Saimiri 342,  $1:10^4$  (4/6 mortality). The irregularity of the titration results is probably due to the considerable variation in virus content of the mosquitoes, the sample of 5 specimens for each pool not being large enough to eliminate this factor.

The titration experiments agree with the experiments involving individual mosquitoes in that there is a rapid loss of virus in the first 48 hours, followed by a slow gain. The gain is so slow during the first 10 days that it is hardly measurable in terms of titer. Yet the ultimate concentration of virus in the mosquitoes may be considerable, as shown by the results obtained with pools of 20- and 36-day-old mosquitoes in these experiments.

*Constant temperature: 20°C.*

In experiments reported in a previous paper (Bates and Roca-García, 1945a) we failed to recover virus from 10 mosquitoes kept for 22 days at 20°, though virus was recovered from 60 per cent of a parallel lot kept at 30°. Once virus was established in the mosquito, however, exposure to 20° seemed to have no adverse effect. In our first experiment using Rodas virus at 20°, we kept a group of mosquitoes for 10 days at this temperature and then transferred them for 10 more days to a constant temperature of 30°; 10 mosquitoes inoculated separately into mice after this treatment all showed virus. A parallel group kept at 30° from the beginning were also 100 per cent infected, and it is probable that these mosquitoes had ingested a considerable amount of virus: serum of the source saimiri killed 3/6 adult mice in the  $1:10^5$  dilution, the highest tested.

We then arranged an experiment with haemagogus lot 197 from which we hoped to determine the rate of loss of virus at 20°, what happened to virus in mosquitoes kept constantly at 20° (did it finally die out, or multiply slowly) and the rate of development of virus in mosquitoes transferred at 10 days from a temperature of 20° to one of 30°. The mosquitoes of this lot fed on Aotus 2 on the 4th day after infection; the animal must have been circulating a tremendous amount of virus, since serum diluted  $1:10^7$  (the highest dilution tested) killed 4/5 adult mice. The "dosage factor" in this experiment would thus be optimum. A parallel group kept constantly at 30° transmitted virus to Aotus 3 ten days after the infectious meal—the shortest incubation period we have encountered.

Five mosquitoes were inoculated separately into mice at 24 hour intervals for the first 5 days; at 10 days 5 more mosquitoes were tested, and the survivors were then divided into 2 lots: 1 lot was transferred to a constant temperature of



TABLE 5

History of virus recovery from mosquitoes of Lot 197 (*Haemagogus capricornii* infected on Aotus 2, 4th day)

DAY	MORTALITY			
	Adult mice*	Per cent	Baby mice	Per cent
Mosquitoes kept at a constant temperature of 20°C.				
0	25/27	93	23/23	100
1	13/27	48	20/24	83
2	13/25	52	19/24	79
3	4/23	17	12/23	52
4	8/28	29	16/23	70
5	1/29	3	7/23	30
10	2/22	9	16/25	64
22	1/30	3	11/20	55

Mosquitoes transferred from constant temperature of 20°C. to constant temperature of 30°C. on 10th day

12	8/26	31	18/25	72
14	23/28	82	20/23	87
16	16/29	55	24/24	100
18	17/28	61	23/23	100
20	20/29	69	19/19	100
22	21/25	84	18/18	100

\* These represent the sum of the mortalities from five mouse groups inoculated on a given day with suspensions of individual mosquitoes.

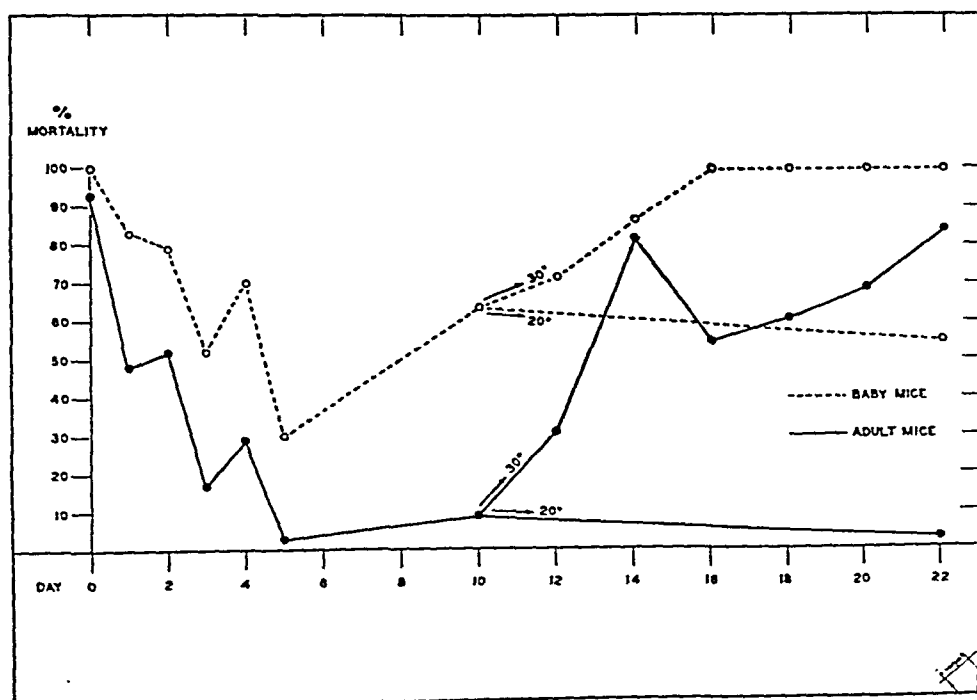


FIG. 2. MOUSE MORTALITIES FROM INOCULATION OF INDIVIDUAL MOSQUITOES OF LOT 197, AT CONSTANT TEMPERATURE OF 20°C. UNTIL 10TH DAY, WHEN PART OF THE GROUP WAS TRANSFERRED TO A CONSTANT TEMPERATURE OF 30°C.

30°, and 5 individuals were tested for virus at 48 hour intervals for the next 12 days; the others were left at 20°, and 5 were tested after 22 days to see whether virus was still demonstrable. The results of this experiment are summarized in table 5; since the mosquitoes were 100 per cent infected, there seems no point in giving the individual results. The mouse mortalities are plotted in graph form in figure 2.

From this experiment, it seems that virus dies out slowly in mosquitoes kept at 20°, reaching a minimum concentration on about the 5th day. It apparently persists at this minimum level indefinitely, since the mosquitoes inoculated on the 5th, 10th and 22nd days showed very similar mouse mortalities. One can imagine that if the original virus dosage ingested by the mosquitoes had been smaller, the virus would have dropped below the threshold detectable with mice and persisted at that level; or that with an even smaller dosage, the virus would have died out during the first 5 days without becoming established in the mosquito at all. When the mosquitoes were transferred to a temperature of 30°

TABLE 6

*Recovery of virus from haemagogus of Lot 298, kept at a constant temperature of 25°C. (per cent mortality from 5 groups of mice inoculated with suspensions of individual mosquitoes on a given day)*

	DAY					
	0	2	4	6	8	10
Per cent mortality in 7-day-old mice.....	100	83	76	95	88	86
Per cent mortality in 45-day-old mice.....	97	4	0	19	30	33

the virus started multiplying, at a rate closely comparable to that shown by lot 181 (fig. 1). These mosquitoes were allowed to feed on baby mice before being killed. There were no transmissions until the 16th day (6 days at 30°) when 2 of the 5 mosquitoes transmitted; 3 of 5 transmitted on the 18th day.

*Constant temperature: 25°C.*

Two experiments were made in which mosquitoes kept at a constant temperature of 25° were tested individually at regular intervals for the presence of virus. The results of the first of these (lot 208, infected on Aotus 3) are summarized in table 6. These mosquitoes ingested a very large virus dose (the aotus serum killed 3/6 adult mice in the dilution 1:10<sup>8</sup>, the highest tested), and the mosquitoes were 100 per cent infected. Virus apparently reached its minimum level in the mosquitoes on the 4th day.

A more detailed experiment was carried out with lot 246, infected on Aotus 11 at a time when the animal was circulating a more moderate amount of virus (2/5 adult mouse mortality with serum diluted 1:10<sup>5</sup>). Five mosquitoes were tested daily for the first 10 days, and at 48-hour intervals for the next 10 days. The results are summarized in table 7. In this case virus was recovered from all of the mosquitoes during the first 2 days, but recovery was more irregular

TABLE 7

*History of virus recovery from daily inoculation of 5 mosquitoes of Lot 246, kept at constant temperature of 25°C.*

DAY	NUMBER OF MOSQUITOES SHOWING VIRUS	MOUSE MORTALITIES*			
		Adults	Percentage	Babies	Percentage
0	5	17/27	63	20/20	100
1	5	26/30	87	20/20	100
2	5	0/24	0	8/16	50
3	2	3/21	14	4/17	23
4	1	0/28	0	1/18	6
5	1	2/28	7	2/18	11
6	5	2/28	7	10/20	50
7	2	0/30	0	4/20	20
8	5	17/29	59	19/20	95
9	5	12/30	40	19/20	95
10	4	5/23	22	12/15	80
12	4	16/24	67	16/16	100
14	5	11/28	39	19/20	95
16	5	20/30	67	18/19	95
18	2	10/12	83	8/8	100
20	3	11/17	65	11/12	92

\* For the 10th day and after, these are based only on mouse groups that included infected animals: i.e., that had been inoculated with mosquitoes known to be infected.

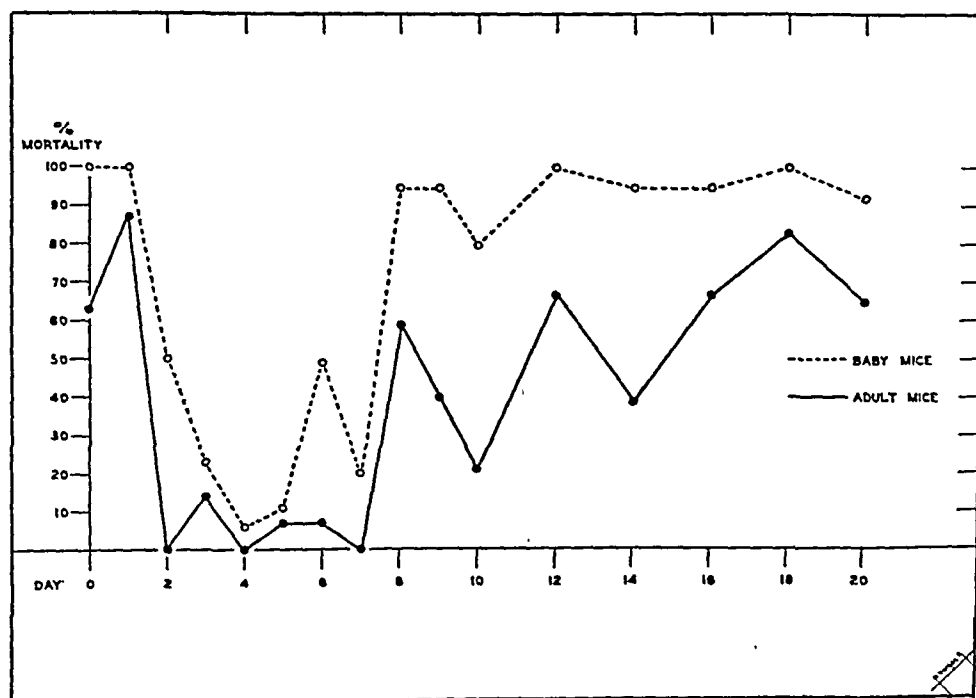


FIG. 3. MOUSE MORTALITIES FROM INOCULATION OF INDIVIDUAL MOSQUITOES OF LOT 246, KEPT AT CONSTANT TEMPERATURE OF 25°C.

subsequently. From the 10th day on, when one would expect the virus in all mosquitoes to have passed above the threshold detectable on mouse inoculation, only 23 of the 30 mosquitoes showed virus (77 per cent). It seems likely that the rest of these mosquitoes had failed to become infected, and in consequence mouse mortalities for this period have been based only on the positive mosquitoes, with the hope of thus giving a better idea of the rate of virus development after establishment in the mosquito. These results are presented in graph form in figure 3. Again the minimum virus level is reached on the 4th day. Unfortunately, no attempt was made to determine the onset of transmission with these mosquitoes.

TABLE 8

*Mouse mortalities from inoculation on alternate days of 5 mosquitoes from Lot 278, kept at 25°C. for 20 hours and 35°C. for 4 hours daily*

	DAY						
	0	2	4	6	8	10	12
Per cent mortality in baby mice.....	100	78	84	100	70	70	100
Per cent mortality in adult mice.....	76	35	55	50	50	57	65

*Alternating temperature: 25°-35°C.*

Results are summarized in table 8 of an experiment with mosquitoes of lot 278, which were kept for 20 hours daily at 25° and for 4 hours daily at 35°. These were infected on Oedipomidas 3 at a time when the animal showed a high titer of circulating virus (2/6 adult mice killed by serum dilution 1:10<sup>7</sup>). Results are very similar to those obtained with a constant temperature of 30°, in that the minimum concentration of virus appears to be on the second day after the infectious meal.

#### IV. THE DOSAGE FACTOR

It is impossible to give a precise evaluation of the dosage factor in these experiments because of the uncertainty of virus titrations and because, in most cases, the number of mosquitoes tested for virus was not large enough to be given statistical significance. A survey of the whole series of experiments, however, gives certain clear impressions that seem to be valid. The experiments are not uniform enough for concise tabular summary from this point of view, and perhaps the best method of review is by the citation of examples. The source animals can conveniently be classified into four groups according to the apparent titer of virus in circulation; haemagogus maintained at a constant temperature of 30° show the following behavior for these four categories of virus dosage:

*Trace of virus* (serum of source animal not infecting adult mice in dilutions greater than 1:10). We have no evidence that haemagogus ever become infected under these circumstances. The best example is lot 204, which fed on Cebus 10 on the 5th day after infection, when the 1:10 serum dilution caused

fatal infection in 2/5 adult mice. No virus was recovered at 20 days from the total suspension of a pool of 11 surviving mosquitoes inoculated subcutaneously in a saimiri monkey.

*Small amount of virus* (serum of source animal not infecting adult mice in dilutions above  $1:10^3$ ). In this category, virus is recovered from an occasional specimen; we have no instance of transmission by bite, but attempts were not made after more than 18 days and the incubation period of the occasional infected haemagogus would presumably be prolonged. Examples: Lot 193, infected on Saimiri 182, serum dilution  $1:100$  causing 1/5 mouse mortality; 1 of 6 mosquitoes showed virus at 17 days. Lot 241, infected on Metachirus 238, serum dilution  $1:100$  causing 2/6 mouse mortality; 1 of 10 mosquitoes showed virus at 17 to 29 days. Lot 189, infected on Saimiri 180, serum dilution  $1:1030$  causing 1/6 mouse mortality; 1 of 11 mosquitoes showed virus at 15 days. Lot 174, infected on Saimiri 108, serum dilution  $1:1000$  causing 2/6 mouse mortality; 3 of 7 mosquitoes showed virus at 14 to 18 days.

*Moderate amount of virus* (serum of source animal causing mouse infections in  $1:10^4$  and  $1:10^5$  dilutions). In this category, virus is recovered from a majority of the mosquitoes and the minimum incubation period is 13 days. Most of the infections of haemagogus on saimiris fall in this class.

*Large amount of virus* (serum of source animal causing mouse infections in the dilution  $1:10^6$  or more). These titers of circulating virus occur occasionally with saimiris, more frequently with aotus. We have always recovered virus from over 90 per cent of the haemagogus infected on such animals, and one is tempted to think that the occasional specimen not showing virus represents a mistake made somewhere in the course of the experiment. The incubation period may be as short as 10 days.

In short, the percentage of mosquitoes showing virus is a function of the amount of virus in circulation in the source animal at the time of feeding, other factors (virus strain, environmental temperature) being constant. In the case of experiments in which only a proportion of the mosquitoes show virus, it would be interesting to know whether the negative mosquitoes are really completely uninfected, or whether their virus content is below the threshold detectable on mouse inoculation. We made two attempts to check this by inoculating saimiri monkeys with the total suspension obtained by grinding individual mosquitoes, on the theory that if any virus was present in such a mosquito the monkey would become infected. In both cases we tested mosquitoes that had been maintained at  $25^\circ$  after feeding on saimiris circulating moderate amounts of virus, since as a general rule only about 50 per cent of the mosquitoes show virus on mouse inoculation under such circumstances. In the first instance 10 mosquitoes were tested in mice at 20 days, and virus was recovered from 7; 4 mosquitoes were then tested at 28 days in saimiris, and all 4 saimiris were infected. In the second instance, 10 mosquitoes were tested in mice at 24 days, and virus was recovered from 6; 5 were then tested at 39 days in saimiris, and 4 of the 5 saimiris became infected. Thus in both cases a higher proportion of the mosquitoes showed virus on saimiri inoculation than did on

mouse inoculation, but the numbers are so small that this result could be accounted for on a chance basis. The failure of 1 saimiri to become infected shows that, in some cases at least, the mosquitoes are probably completely free of virus. In some cases, however, failure to recover virus from mosquitoes on mouse inoculation surely means simply that the virus is below the threshold detectable by this means.

It is interesting to speculate as to why certain individual mosquitoes in a given experiment become infected, or show demonstrable virus, while others do not. The most likely explanation would be the varying amounts of virus ingested by the mosquitoes at the time of feeding: those engorging fully would acquire a larger absolute number of virus particles than those taking less blood. This does not, however, seem to be the entire explanation. In one experiment, we divided the mosquitoes into 2 lots after they had fed. One lot included only specimens whose abdomens had become distended with blood; the other, specimens in which the abdomen had not obviously become distended. After 22 days at 30°, 10 specimens of each group were tested in mice and virus was recovered from 9 specimens in each case: the mouse results were closely similar in both cases, the positive mosquitoes showing large amounts of virus.

It seems entirely possible that the factor of mosquito strain enters in such cases: that some individual haemagogus are more susceptible to infection than others. Where an overwhelming dosage is ingested and the mosquitoes are kept under optimum temperature conditions, they all become infected; but where the virus dosage is smaller, and the temperature conditions less favorable, individual differences in susceptibility can enter in. This hypothesis could be tested with mosquito species that can be maintained as laboratory colonies so that genetic lines could be separated out. This would be analogous with the situation described by Huff (1934) for *Culex pipiens* and plasmodium infection.

#### V. THE VIRUS STRAIN FACTOR

Our success in maintaining laboratory transmissions of yellow fever virus with the Perez and Rodas strains of virus contrasted strongly with the partial or complete failure of our experiments during previous years. Undoubtedly, two important factors in the later success were the maintenance of the mosquitoes at relatively high temperatures, and the improved entomological techniques that resulted in a greater mosquito longevity. We thought, however, that the use of new virus strains might have been an additional factor. Our early work was carried out with strains that had been subject to considerable laboratory manipulation; they had, in particular, been passaged several times through mouse groups, and had been maintained for prolonged periods in a desiccated state. We thought it worth while to check the possible effect of mouse brain passage on virus infectivity for mosquitoes.

We made 3 separate series of experiments in which "pantropic" virus of the regular haemagogus cycles was passaged through 6 mouse groups and then inoculated into a saimiri, which was used as a source animal for haemagogus infections. The virus acquired a "fixed" character in the first 3 or 4 brain

passages, the mouse mortality becoming regular, with death on the 6th and 7th days, in contrast with the irregular mortality and prolonged incubation period of the unmodified virus.

Two such experiments were made with Perez virus and 1 with Rodas virus. The mosquitoes were kept at 30° in all cases. In the first experiment 62 haemagogus fed on the saimiri, and 25 of these were tested individually for virus from 12 to 29 days later, without virus being recovered in any case. The titer of virus in circulation in the monkey must have been low, as the mosquito "controls" (inoculated directly after engorging) infected only about half of the test mice: the titration of the monkey's serum was incomplete. Still it seemed remarkable that virus was recovered from none of the 25 mosquitoes.

Data on the second experiment are more complete. Perhaps the best method of summary is by comparing the results of the experiment with mouse adapted virus (Lot 176) with those of an experiment with unmodified virus (Lot 172) that was carried out at the same time under the same conditions.

Serum titrations of the source monkeys were:

Serum dilutions.....	1:10	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>
Saimiri 108 (unmodified virus).....	4/6	1/6	3/5	3/5	0/6
Saimiri 120 (mouse adapted virus).....	6/6	6/6	5/6	1/6	0/6

Adult mouse mortalities from control mosquitoes:

Lot 172 (unmodified virus).....	3/6	3/5	4/5	3/6
Lot 176 (mouse adapted virus).....	5/6	3/6	5/6	

Transmission to baby mice, 13 days and more after infectious meal:

Lot 172 (unmodified virus):	7 fed, 4 transmitted
Lot 176 (mouse adapted virus):	7 fed, none transmitted

Recovery of virus by mouse inoculation:

Lot 172 (unmodified virus):	18 of 20 mosquitoes showed virus (90 per cent)
Lot 176 (mouse adapted virus):	1 of 12 showed virus (8 per cent)

The problem here is to decide how much the difference in the behavior of the 2 mosquito groups is due to dosage ingested, and how much to difference in virus strain. The 2 titrations show nicely the difference in behavior between unmodified and modified virus: if we had an objective method of measurement, it would probably be found that Saimiri 108 was circulating at least 10 times as much virus as Saimiri 120; and the difference falls on the dividing line between our categories of "small" and "moderate" amounts of virus, which seems to be very significant in governing haemagogus infection, as pointed out in the preceding section.

In the 3rd experiment, 161 haemagogus fed on a saimiri infected with modified Rodas virus (6 brain passages). Serum of the source monkey infected 4/6 adult mice in the 1:10<sup>3</sup> dilution, 0/6 in the 1:10<sup>4</sup> dilution. It thus again was on the borderline between the categories of "small" and "moderate" virus amounts. In this experiment we attempted to follow the history of the virus by the technique used in the temperature experiments, testing 5 separate mos-

quitoes daily for virus on the first 10 days, and on alternate days thereafter. The virus disappeared rapidly, only 1 mosquito being positive after 24 hours. After the 5th day, virus was recovered from 9 of 57 mosquitoes (16 per cent), positive mosquitoes showing a completely random distribution in time. Twenty-one attempts at transmission by bite to baby mice were made between the 12th and 23rd day. Two mosquitoes transmitted, both on the 21st day; both showed considerable virus on mouse inoculation (infecting 6/6 adult mice). It is interesting that the virus continued to show enhanced neurotropic properties even after monkey and mosquito passage, since the incubation period in test mice was generally much shorter, and mortality more regular, than in the case of unmodified virus.

From these 3 experiments one conclusion can surely be drawn: that it would be very difficult if not impossible to establish cyclic transmissions with *haemagogus* mosquitoes using a strain of virus that had been passaged several times through mouse brain. Saimiri monkeys infected with such virus show relatively low titers of virus in circulation (even though the infections are usually fatal). With the evidence at hand, we cannot say how much the low infection rate of *haemagogus* is due to this dosage factor, and how much to modification of the adaptive properties of the virus that enable it to invade the mosquito host, though we have the impression that both factors are probably involved.

#### VI. VIRUS TRANSMISSION

Transmission experiments with yellow fever (and other viruses) have generally been made by allowing a group of mosquitoes to feed on a susceptible host, so that there is no way of knowing how many of the mosquitoes were actually infective. The susceptibility of baby mice to subcutaneous inoculation provides an animal that can be used for testing transmission by individual mosquitoes, as pointed out by Bugher (1941). Our first experiments (Bates and Roca-García, 1945a, table 2) indicated that there might be considerable variability in the infectiveness of individual *haemagogus* mosquitoes, and we consequently planned a more detailed study of this phenomenon. A series of parallel tests indicated that the susceptibility of baby mice to our strains of virus by the subcutaneous and intracerebral inoculation routes was closely similar, so that it seems probable that these mice are sensitive indicators of transmission. The chief difficulty in handling baby mice is that sick individuals are sometimes eaten by the mother. We have had no losses except where there has been strong reason to presume that the baby was infected; nevertheless such cases have not been counted as "transmissions" in crucial experiments. In the vast majority of cases, including all crucial experiments (e.g., defining minimum incubation periods), the sick baby mouse has been recovered and brain material passaged to confirm the nature of the infectious agent.

As was pointed out in the section on "dosage" above, the amount of virus originally ingested by the mosquitoes has considerable influence on the mosquito incubation period. Studies of the effect of temperature on the time required for transmission are thus most satisfactory if carried out with parallel



lots of mosquitoes infected on a given host at the same time. Our best experiment of this sort was based on a group of haemagogus that fed on *Aotus 15* at a time when considerable virus was in circulation (serum killing 4/5 adult mice in the dilution 1:10<sup>6</sup>, the highest tested). These mosquitoes were divided at random into 5 lots of 44 mosquitoes each: 3 lots were kept at constant temperatures of 25°, 30° and 35° respectively; 1 for 20 hours daily at 25° and 4 hours daily at 30°; and the last for 20 hours daily at 25° and 4 hours daily at 35°. The results are summarized in table 9.

In experiments in which a group of mosquitoes feed on a susceptible host, the first transmission would occur when the first mosquito became infective: even though 20 mosquitoes feed, it may be that only one is infective at that time. There seems, in fact, to be considerable individual variation in the time required for a mosquito to become infective, and in all of our experiments, we have observed that the longer the elapsed time, the higher the proportion of infective mosquitoes. One case will suffice to illustrate the phenomenon. The haema-

TABLE 9

*Transmission to baby mice by haemagogus (infected on Aotus 15 and kept under different temperature conditions)*

LOT NUMBER	TEMPERATURE	TOTAL NUMBER OF FEEDINGS	DAY OF FIRST FEEDING	FIRST TRANSMISSION	TOTAL NUMBER OF TRANSMISSIONS
				days	
271	25°	58	15	28	4
272	25°-30°	59	10	23	14
273	25°-35°	54	10	12	12
274	30°	18	10	10	13
275	35°	4	6	none	

gogus of lot 277, infected on *Oedipomidas 3* and maintained at 25° for 20 hours daily and 35° for 4 hours daily, were kept for 60 days, during which time 84 tests of transmission were made with baby mice. The results, grouped in 5-day periods, are summarized in table 10. The first transmission occurred on the 12th day after the infectious meal, but the majority of the mosquitoes did not transmit until the 20th day or later. In this case, in a parallel lot kept at a constant temperature of 30°, the first transmission was also on the 12th day.

We kept records on individual mosquitoes in all of these experiments, so that it is possible to follow the history of a given individual. The selection of the mosquitoes for feeding tests was generally random—the mosquitoes frequently showed no desire to bite, and they would be tested with a given baby mouse until an individual was found that would insert its proboscis. If a given mosquito probed a baby mouse, the act was regarded as a "transmission attempt" regardless of whether blood was drawn or not. Thus transmission records are frequently available on a given individual mosquito over considerable periods of time. Once an individual becomes infective, it remains infective for life. Thus mosquito no. 39 of lot 272 failed to transmit at 12 and 21 days; but it

transmitted at 23, 24, 25, 26, 28 and 32 days. Mosquito no. 18 of lot 277 transmitted at 12, 24, 32, 33, 34, 36 and 37 days—every occasion on which it was tested. Very rarely a mosquito that had once transmitted failed to infect a baby mouse on some subsequent occasion: thus mosquito no. 6 of lot 277 failed to transmit at 16, 23, 26, 29 and 32 days, but transmitted at 34, 36, 37, 48 and 50 days; at 51 days it is recorded as biting a baby mouse without transmitting. We can only find 2 other such instances in the many hundreds of transmission attempts.

## VII. DISCUSSION

From the experiments reported in the present paper it appears that the history of the yellow fever virus in the mosquito falls into two periods—a period of virus loss and a period of virus gain. This is in accord with what would be expected on theoretical grounds, and with various previously published experiments, notably those of Whitman (1937). Whether the mosquito becomes infected or not apparently depends on the events of the period of virus loss, which are presumably centered in the gut of the mosquito: it is probably a question of whether the virus can become established in the mosquito tissue (i.e., penetrate the gut wall) before it dies or has been eliminated. With a given mosquito strain and a given virus strain, the course of events in the period of virus loss seems to be governed by two variable factors: the amount of virus originally ingested by the mosquito and the environmental temperature. The more virus, the better the chance that some will get established in the mosquito tissues. The higher the temperature (within the range tested), the faster the course of events; and apparently also, the better the chance that some virus will get established.

The period of virus loss apparently lasts for 2 days at 30°, 3–4 days at 25° and 5 days at 20°. The virus particles in the gut would be in a non-living suspension, so that there would presumably be no multiplication; the loss in virus during this period would thus seem to reflect the rate of death of virus particles at these temperatures and in this medium. It is curious, under these circumstances, that the proportion of mosquitoes becoming infected with a given dosage and given virus strain should depend directly on temperature. Evidence on this point was presented in a previous paper (Bates and Roca-García, 1945a), and later experiments, especially those involving moderate virus dosages, show the same phenomenon. The phenomenon is not confined to yellow fever virus and haemagogus mosquitoes. Milzer (1942) found that *Aedes aegypti* did not become infected with the virus of lymphocytic choriomeningitis at temperatures below 26°, and it seems likely that many of the contradictory results in published insect-virus experiments stem from differences in the temperature factor. The increased likelihood of infection at higher temperatures may be a property of the mosquito, the virus or both: it may reflect an increased permeability of the mosquito gut at higher temperatures, or an increase in some activity property of the virus particles themselves.

In the period of virus gain, the relationship between rate of virus multiplica-

tion and temperature is surely simply another example of the influence of temperature on the speed of biological processes, but the data are too incomplete to warrant any detailed analysis from this point of view. It seems to us that infectiveness (ability to transmit) in the mosquito may be threshold phenomenon, dependent on the level of concentration of virus in the mosquito. Such evidence as there is suggests that yellow fever and other viruses show no tissue specificity in the mosquito host (Davis and Shannon, 1930; Merrill and Ten-Broeck, 1934), though this is a problem that has hardly been investigated in sufficient detail. The appearance of virus in the salivary secretion of the mosquito might then be dependent simply on the total virus content of the insect. This would explain the great variability in the "incubation period" (time between the infectious meal and the first infective meal) in individual mosquitoes of the same lot (e.g., the data in table 10).

TABLE 10

*Transmission by bite to baby mice (haemagogus of Lot 277, infected on Oedipomidas 3 and maintained at 25°-35°C.)*

DAYS	NUMBER OF FEEDINGS	NUMBER OF TRANSMISSIONS	PER CENT TRANSMITTING
11-15	20	3	15
16-20	17	2	13
21-25	12	7	58
26-30	11	6	55
31-35	12	10	83
36-60	12	11	92

The titrations of virus content of *Aedes aegypti* reported by Whitman (1937) seemed to show that the amount of virus in the mosquito did not increase indefinitely—that after an initial period of regular increase it was subject to considerable and irregular variation. If this phenomenon occurs in haemagogus, it would not be demonstrable from our experiments, since we did not make many titrations with long-infected mosquitoes.

At 20° there seems to be no demonstrable increase in the virus content of the mosquito over a period as long as 22 days (data in table 5) and it is likely that mosquitoes would never become infective at this temperature. At the other extreme, at a constant temperature of 35°, experiments were not satisfactory because of the high mortality of the mosquitoes, the majority dying within the first few days: it was also difficult to induce mosquitoes kept at this temperature to feed. Twenty-eight transmission attempts were made with 3 different lots, from 5 to 12 days after infection, with a possible transmission once at 12 days (not confirmed by passage). In some cases mosquitoes failed to transmit after 10 days at 35°, when transmission was obtained from parallel lots kept at 30°. At constant temperatures of 25° and 30° the minimum incubation periods observed have been 28 days and 10 days respectively. These results, it may be noted, differ considerably from those usually reported for *Aedes aegypti* and the Asibi strain of virus (Davis, 1932), being much longer. Davis also was able to

carry out experiments at temperatures above 30°, obtaining transmission after 4 days at 36°. From our data it is impossible to determine how much the lengthening of the incubation period is a property of the mosquito species and how much a property of the virus strain, though probably both are involved, as was pointed out in our first article.

We were greatly interested in carrying out experiments at varying temperatures, since temperature conditions in the forest canopy, where haemagogus are found most abundantly, are subject to wide diurnal fluctuations. It is interesting in this connection that virus development in mosquitoes kept for 20 hours daily at 25° and 4 hours daily at 35° was almost as rapid as in mosquitoes kept at a constant temperature of 30°. The difference between 4 hours daily exposure to 30° and 4 hours daily exposure to 35° is particularly striking (table 9; other experiments gave similar results). The difference in "mean temperature" between these is slight, since the daily mean calculated by hours in the one case would be 25.8° and in the other 26.6°. The accelerating effect on virus development must be due to the short daily exposure to the (for a mosquito) very high temperature of 35°.

We have made a number of experiments designed to study the effect of various temperature conditions on the longevity, speed of development and physiology of haemagogus mosquitoes. These will be written up separately. Of interest in the present connection is the fact that the 20 hours daily exposure to 25° and 4 hours daily shift to 35° seems to be very favorable to the mosquitoes. The longevity is greater than at a constant temperature of 30°; and the per cent of females laying eggs and the number of eggs laid per female are both greater than at 30°, taking these as indexes of the favorableness of temperature conditions for physiological processes in the mosquito.

In our first article (Bates and Roca-García, 1945a) we pointed out the relation between laboratory findings of the effect of temperature on virus development, and field findings of relatively high diurnal temperatures in the canopy zone of the forest. The favorable effect of short daily exposure to 35° is an even more striking example of this relation. Shade temperatures of 35° are rarely found in the Villavicencio area, but it is a common observation that haemagogus mosquitoes are to a surprising degree sun-loving insects. They are apt to bite commonly even in the forest floor zone in open spots where the sun reaches the ground; and the canopy zone, where they are abundant, contains considerable areas of sunlight. Various investigators have found that the body temperature of an insect rises rapidly in sunlight. For example, Wigglesworth (1939, p. 359) in his summary of the literature on this subject, quotes Strelnikow, who found that the temperature of *Bombus* rose from 28.7° in the shade to 41.6° in the sun in the course of 5 minutes. We have no data on the possible daily body temperatures of haemagogus mosquitoes in their natural environment, but it does not seem unlikely that they would be exposed for short daily periods to relatively high temperatures. Results with the 25°-35° alternation are certainly much more likely to approximate natural conditions than results with a constant temperature of 30°, which can only have a theoretical interest.

## VIII. SUMMARY

1. The effect of temperature on virus development in haemagogus mosquitoes was studied by the inoculation in mouse groups of individual mosquitoes at regular intervals after the infectious meal and by the titration of pools of mosquitoes. It was found that there was an initial period of virus loss, followed by a period of virus gain, the rates in both cases depending on the temperature. The period of virus loss lasted for 5 days at 20°, 3–4 days at 25° and 2 days at 30°. At 20° the level of virus seemed to remain stable after the period of loss, there being no demonstrable increase over a 22 day period. At higher temperatures the rate of gain seemed to be a direct function of the environmental temperature.

2. The percentage of mosquitoes becoming infected and the length of the incubation period seemed also to be a function of the amount of virus ingested with the infectious meal. On the basis of titer of virus in circulation at the time of feeding, experiments can be divided into 4 arbitrary categories: trace of virus (serum of source animal not infecting adult mice in dilutions greater than 1:10); small amount of virus (no infections in dilutions above 1:10<sup>3</sup>); moderate amount of virus (no infections in dilutions above 1:10<sup>5</sup>) and large amount of virus (infections in dilution of 1:10<sup>6</sup> or more). In the first category, virus has in no case been recovered from haemagogus; in the second, occasional individuals become infected; in the third, the majority of the mosquitoes show virus; in the fourth, virus is regularly recovered from 90 per cent or more of the mosquitoes. The minimum incubation period after the ingestion of a "moderate amount of virus" is 13 days at 30°. This may be shortened to 10 days where a "large amount of virus" has been ingested. There is some evidence that infection at a given temperature and given virus dosage depends in part on the characteristics of the individual mosquito.

3. Experiments were undertaken with pantropic virus strains modified by serial passage (6 consecutive passages) in mice. It was difficult to infect haemagogus on saimiri monkeys inoculated with these modified virus strains; with the evidence at hand it is impossible to decide how much this was due to the lower titers of virus circulated by such monkeys, and how much to possible modification of the ability of the virus particles to invade mosquito tissue.

4. Attempts were made to define the incubation period in mosquitoes by large numbers of tests for transmission with individual mosquitoes using 3-day-old mice as test animals. It was found that there was considerable variation among individual mosquitoes of the same lot in the time at which they became infective, but that once a mosquito became infective it remained so for life. The minimum incubation period was found to be 28 days at 25°; 23 days in mosquitoes kept for 20 hours daily at 25° and 4 hours daily at 30°; 12 days for a similar alternation of 25°–35°; and 10 days at a constant temperature of 30°. Results were unsatisfactory at a constant temperature of 35°, but no transmissions were obtained in 28 attempts at periods between 5 and 12 days.

5. The very favorable results obtained with mosquitoes alternated between 25° (20 hours) and 35° (4 hours) suggest that relatively short exposures to high temperatures in nature may greatly accelerate virus development.

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# AN EXPERIMENT WITH NEUROTROPIC YELLOW FEVER VIRUS IN SAIMIRI MONKEYS AND HAEMAGOGUS MOSQUITOES<sup>1</sup>

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In the course of our experiments with the transmission of yellow fever by haemagogus mosquitoes we became interested in the possible effect of mouse brain passage on the ability of a given virus strain to invade mosquito tissues. In a previous paper (Bates and Roca-García, 1946b) we have described experiments in which "pantropic" virus strains were tested in saimiri monkeys and haemagogus mosquitoes after a series of six mouse brain passages; mosquitoes rarely became infected with such virus, but it was difficult to determine how much this was due to change in the mosquito adaptations of the virus and how much to the reduced titer in circulation in monkeys inoculated with such passaged strains. We decided, in this connection, to try a few experiments with completely "fixed" neurotropic virus, using the strain currently employed in protection test routines—"New York Standard Virus Lot 40," the 250th mouse brain passage of French neurotropic virus. We found, from exploratory experiments, that saimiri monkeys generally showed only a low titer of virus in circulation after subcutaneous inoculation with this strain, so we decided to see whether the titer in circulation could be stepped up by serial passage in these monkeys. We thought it would, in itself, be interesting to see whether the fixed virus showed demonstrable modification after a number of monkey passages.

The methods that we employ in handling saimiri monkeys and haemagogus mosquitoes have been described in previous articles (Bates, 1944; Bates and Roca-García, 1945). In our calculations of titers and dosages in the present study we used the standard 50 per cent end-point method of Reed and Muench (1938).

## BEHAVIOR OF NEUROTROPIC VIRUS IN SAIMIRIS

The saimiri passages were started by inoculating Monkey no. 266 intramuscularly with 0.03 cc. of a 1:150 dilution of rehydrated mouse brain suspension of "Standard Virus New York 40"; the control titration in mice showed that this amount of inoculum contained 159 mouse m.l.d. The monkey was bled daily from the 3rd day, and the serum was titered by the inoculation of serial dilutions in groups of white mice. Exploratory experiments had shown that the maximum titer in circulation generally occurred on the 4th day, so this interval was adopted as routine for passage. The second saimiri was inoculated intra-

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muscularly with 0.03 cc. of a 1:10 dilution of the 4th day serum of Saimiri 266; mouse titration showed this inoculum to contain 2,340 mouse m.l.d. of virus. The same procedure was followed routinely through 22 saimiri passages, when the experiment was discontinued.

Ths histories of these animals are summarized in table 1. Nine of the 22 survived infection; 1 (No. 308) was killed (bled to death to obtain serum for desiccation); 1 (No. 309) died on the 3rd day, the early death perhaps being related to an intense parasitism with *Acanthocephala*; and the remaining 11 ap-

TABLE 1

*Historics of saimiris used in serial passage experiment with neurotropic virus*

PASSAGE NO.	SAIMIRI NO.	DOSAGE INOC.	CIRCULATING VIRUS: DAY					TITER ON 4TH DAY	DAY OF DEATH
			3	4	5	6	7		
1	266	159	+	+	+	+		$1:2.23 \times 10^4$	7
2	265	2,340	+	+	+	-		$1:1.0 \times 10^3$	7
3	268	100	+	+	+			$1:2.52 \times 10^5$	6
4	271	25,200	+	+	-	-	-	$1:4.61 \times 10^1$	S
5	269	5	+	+	+	+		$1:4.5 \times 10^4$	6
6	277	4,500	+	+				$1:4.5 \times 10^5$	5
7	276	45,000	+	+	-			$1:5.75 \times 10^1$	5
8	278	6	+	+	+			$1:1.28 \times 10^4$	5
9	282	1,280	+	+				$1:5.10 \times 10^5$	4
10	287	51,000	+	+	+	-	-	$1:1.80 \times 10^2$	S
11	288	18	+	+	+	+	+	$1:4.38 \times 10^3$	S
12	289	438	+	+	+			$1:1.52 \times 10^4$	6
13	290	1,520	+	+	+	-	-	$1:2.51 \times 10^4$	S
14	291	2,510	+	+	+	-	-	$1:4.2 \times 10^3$	7
15	292	420	+	+	+	-	-	$1:2.18 \times 10^2$	S
16	293	21	+	+	+	+	tr.	$1:1.0 \times 10^3$	S
17	264	100	+	+	+	+	tr.	$1:3.2 \times 10^1$	S
18	296	3,200	+	+	+	-	-	$1:4.0 \times 10^2$	S
19	304	40	+	+	+	+	-	$1:8.9 \times 10^3$	S
20	309	890	+				>	$1:1.0 \times 10^4$	3
21	308	+1,000	+	+				$1:1.0 \times 10^2$	Killed
22	311	10	+	+				$1:1.0 \times 10^4$	4

parently died as a result of the virus infection. Signs of stomach haemorrhage were found in 6 of these 11 animals; histological examination of liver tissue was made in all cases, but in no instance were lesions characteristic of yellow fever found. Six of the 22 animals showed slight alteration of the normal temperature rhythm, which might be interpreted as febrile reaction, but in most cases body temperature remained perfectly normal except for a rapid drop 12 to 24 hours before death in the fatal cases. Unfortunately we were not in a position to make histological examinations of the brain material for possible encephalitic lesions; the animals did not show any obvious paralysis. The virus showed no demonstrable change in behavior on white mouse inoculation in the course of these passages: the incubation period in mice continued to be short and regular, char-

acteristic of the fixed virus. There was also no regular increase in titer of circulating virus in the successive saimiris. If anything, the virus seemed to lose virulence for saimiris, since 8 of the first 9 animals died, in contrast with 3 or 4 (depending on the diagnosis of saimiri 308) of the next 13. Certainly the virus did not show any measurable shift from "neurotropism" to "viscerotropism" in the course of these passages, which confirms the general impression that the change is not regularly reversible.

It is interesting to compare this series with a similar passage experiment made with the "pantropic" Rodas strain of virus (Bates and Roca-García, 1946a, table 4). The pantropic virus was carried through 10 passages only; in comparison with the first 10 passages of neurotropic virus, the mortality rate and day of death is closely similar, as is the incidence of stomach haemorrhage in the fatal cases. In neither series were characteristic liver lesions present. In both series the maximum titer of virus was generally on the 4th day, occasionally on the 3rd or 5th; but the amount of virus in circulation was much greater in the pantropic series, and there was generally a febrile reaction.

Lloyd and Penna (1933) have reported a series of experiments with rhesus monkeys inoculated with neurotropic virus by extraneural routes. The infection in rhesus was in all cases much less severe than in saimiris, being in no case fatal, and the virus repeatedly failed to survive after a few monkey passages.

#### ATTEMPTS TO TRANSMIT NEUROTROPIC VIRUS WITH HAEMAGOGUS MOSQUITOES

The saimiri monkeys of the serial passage experiment were used as source animals for 8 infection experiments with haemagogus mosquitoes. The histories of these experiments are summarized in table 2. All attempts to recover virus by inoculation of mosquitoes into mice, by inoculation of pools of mosquitoes into monkeys and by feeding mosquitoes on baby mice or on saimiri monkeys were negative, with one exception. The exception is Saimiri 316, inoculated with the total suspension of a pool of 4 mosquitoes of lot 261, 19 days after infection. This monkey showed a somewhat delayed infection, with circulation virus on the 3rd through the 7th day (not bled subsequently), with maximum temperature on the 6th day and death on the 9th day. The mice inoculated with serum from this animal showed the prolonged incubation period characteristic of a pantropic virus strain. This appears surely to be an accidental infection, but if so, it is the only such infection that we have had in hundreds of monkeys handled in the course of these transmission experiments.

The possible explanations are: (1) a confusion of animals or records. This seems to be ruled out, as there was no possible animal present in the inoculation room at that time with which a switch could have occurred. (2) A "natural" infection in the mosquitoes: these mosquitoes were brought in from the field, and 1 of them might have had virus acquired before capture. We have no precedent for this (but it would not be apt to occur frequently); there is, however, no evidence that yellow fever was present anywhere in the Villavicencio region during 1945, which makes this explanation unlikely. (3) That mosquitoes were switched. There were many infection experiments being carried out at the same

time with pantropic "Rodas" virus, and it seems most probable that an individual from one of these experiments somehow got into the neurotropic experiment. We have tried to guard against such a possibility, but with hundreds of mosquitoes being handled separately and routinely, such an accident might well happen. (4) If the virus really came from the neurotropic experiment, it is a unique case of virus recovery, and the virus was completely changed in the course of the single mosquito passage, reverting on mouse inoculation to the incubation period characteristic of pantropic strains. This would seem extraordinary, but not beyond the bounds of possibility. Unfortunately we can-

TABLE 2

*Haemagogus* infection experiments with neurotropic virus  
(All mosquito lots kept at a constant temperature of 30°C.)

INFECTION DATA						ATTEMPTS AT VIRUS RECOVERY, 15 DAYS OR MORE AFTER INFECTION (NEGATIVE EXCEPT LOT 261)			
Haemagogus lot no.	Saimiri no.	Titer at time of feeding	Mortality of controls		No. feeding	Inoculation in mice		Inoculation in Saimiri	Attempts at transmission
			No.	Total mortality		Killed mosquitoes	Dead mosquitoes		
236	276	1:58	5	2/29	50	2			
237	287	1:180	3	0/16	60	4			
244	289	1:120,000	3	17/17	45	3	3		6 feedings on baby mice
245	289	1:15,200	3	12/17	49	15			12 feedings on baby mice
249	292	1:218	5	11/28	88	4		Pool of 8	4 feedings on baby mice
									5 feedings on saimiri
257	304	1:4,500	5	19/29	66	7	8		
258	304	1:8,900	5	29/29	58	7	7	Pool of 4	9 feedings on baby mice
261	311	1:7,200	4	23/24	47		8	Pool of 4*	11 feedings on baby mice

\* Inoculated into Saimiri 316, which showed an infection characteristic of pantropic virus; it is probable that a mistake was made with these mosquitoes.

not attempt to repeat the experiment, since the program of transmission work has been suspended.

From these experiments, it appears that fixed neurotropic virus is completely non-infective for haemagogus mosquitoes, even after repeated passage through saimiri monkeys. In experiments 236, 237 and 249 the titer of circulating virus was so low that one would in no case expect mosquito infections, but the monkeys used as source animals for experiments 244, 245, 258 and 261 had a high enough titer of circulation virus so that one would expect to find at least a few of the mosquitoes infected. There is, of course, always the difficulty of making titration comparisons between mouse-adapted and pantropic strains of virus, but the results of these experiments are so uniformly negative (always with the exception of Saimiri 316 from mosquito lot 261) that it would seem possible to rule this dosage factor out.

## DISCUSSION

Davis, Lloyd and Frobisher (1932) found difficulty in infecting *Aedes aegypti* on rhesus monkeys circulating virus of a fixed neurotropic strain. They were able to obtain some mosquito transmissions, in contrast with our complete failure to infect *Haemagogus capricornii* under comparable conditions, but this doubtless reflects the much greater susceptibility of *Aedes aegypti* to virus infection in general. Whitman (1939) found that *Aedes aegypti* under certain circumstances (immersion of larvae in mouse brain suspension) may become infected with the highly modified virus of yellow fever vaccine (strain 17D), though no successful transmissions were obtained.

The phenomenon of a loss of infectiousness for mosquitoes associated with a gain in neurotropism in a particular virus strain seems not to be confined to yellow fever. Hammon and Reeves (1943), for instance, attribute their success in obtaining laboratory transmission of St. Louis encephalitis, in contrast with previous failures, in part to the "use of a virus freshly isolated from mosquitoes, not brain passage fixed." Sabin and Schlesinger (1945) found with dengue virus after mouse passage that "*Aedes aegypti* . . . became infected with difficulty since large numbers of mosquitoes and an extrinsic incubation of more than three weeks were required to transmit the virus."

There is probably in all of these instances an effect due to the decreased titer of circulating virus in the case of mouse passaged strains, resulting in a reduction of the virus dosage picked up by the mosquitoes. The increase in neurotropism, in other words, is generally associated with a decrease in the amount of virus liable to appear in peripheral circulation in susceptible hosts. But this explanation seems inadequate to account for all of the results, particularly for the complete failure to infect haemagogus mosquitoes with saimiri-passaged neurotropic virus reported in the present paper.

## SUMMARY

A fixed neurotropic strain of yellow fever virus, in its 250th mouse brain passage, was maintained in saimiri monkeys for 22 serial passages by the intramuscular inoculation of a 1:10 dilution of the 4th-day serum of the preceding animal. The response of the monkeys to infection was similar to that observed in passage experiments with pantropic strains. Eleven of the 22 apparently died as a result of virus infection; 6 of the 11 showed signs of stomach haemorrhage; in no instance did postmortem liver tissue show pathological lesions characteristic of yellow fever; in no instance was there a well-defined febrile reaction. The virus showed no demonstrable change in behavior after intracerebral inoculation in white mice in the course of the passage experiment: the mouse incubation period continued short and regular, characteristic of the "fixed" virus. There was no regular increase in titer of circulating virus in monkeys in successive passages; the strain seemed to lose virulence, since 8 of the first 9 infections were fatal in contrast with 3 of the following 13.

Eight attempts to infect haemagogus mosquitoes on these monkeys failed completely: virus was not recovered by inoculation of the mosquitoes into mice

or into saimiris, or by feeding them on baby mice or saimiris except in one instance which was probably due to a confusion of mosquitoes infected with another strain of virus.

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# THE LABORATORY TRANSMISSION OF YELLOW FEVER VIRUS BY HAEMAGOGUS SPLENDENS\*

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## INTRODUCTION

Interest in mosquitoes of the genus *Haemagogus* as vectors of yellow fever was aroused by the demonstration of the importance of *Haemagogus spegazzinii falco* (see (2) Kumm et al., 1946) in the epidemiology of jungle yellow fever in Colombia by Bugher, Boshell-Manrique, Roca-García, and Osorno-Mesa (1).

Although *Haemagogus spegazzinii falco* is the only important vector of this genus recognized at the present time, one is naturally interested in knowing the vector potentialities of other species of the genus that have been found in the endemic areas. The recent article by Kumm, Osorno and Boshell (2) gives data on the known distribution of *Haemagogus splendens* in Colombia, and supplementary information is available in a paper by Osorno (3). Recently, Waddell and Taylor (4) have obtained transmission by *Haemagogus equinus*, a species known to occur in endemic yellow fever zones.

The present report describes experiments showing the transmission of yellow fever by *Haemagogus splendens*.

## MATERIALS AND METHODS

The methods used in these experiments are so similar to those used by other investigators that they need not be described in detail. Adequate descriptions are available in the articles by Waddell and Taylor (4), and Bates and Roca (5).

## MOSQUITOES

All of the experiments described below were performed with laboratory-reared *Haemagogus splendens*. The management of a colony of this species has been described by Osorno (3). During the course of these experiments, no other infected mosquitoes were maintained in the laboratory.

In order to obtain information with regard to the effect of virus titer on the percentage of infected mosquitoes, only mosquitoes that engorged were kept; those not engorging were discarded immediately after having had an opportunity to feed. Mosquitoes feeding at a given time were maintained in separate cages and never mixed with any other group.

## VIRUS STRAIN

Only the "Chichimene" strain of yellow fever virus was used. This strain was selected principally because it was a local strain and showed marked neurotropism

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for white mice. The strain was originally isolated in a rhesus monkey infected by the bite of wild mosquitoes. It was then passaged once intracerebrally in white mice and reinoculated into a rhesus monkey, the dried serum of the latter serving as the source of virus in these experiments. A detailed description of the isolation of this strain may be found in the paper by Bugher et al. (1).

#### MONKEYS

Two species of local monkeys were used in the experiments, the saimiri monkey (*Saimiri sciureus caquetensis* Allen) and the douroucouli (*Aotus trivirgatus*). The monkeys<sup>1</sup> were all obtained from the Villavicencio area. A discussion of the nomenclature of the two types may be found in articles by Bates (6), and Bates and Roca (7). Only animals whose sera gave negative results in pre-experimental protection tests were used.

#### MOUSE TECHNIQUES

All tests for the presence of yellow fever virus in mosquitoes were made by subcutaneous inoculation of the mosquito suspension into 3- to 5-day-old mice, according to the method described by Bugher (8). Tests for circulating virus and the estimations of titer were all carried out in adult white mice inoculated intracerebrally, following the suggestions of Theiler (9). The sera were tested for the presence of yellow fever antibodies by the intracerebral method in accordance with the technique adopted by Bugher (10).

#### CALCULATION OF TITERS

All virus titers were calculated by the fifty per cent end point method recommended by Reed and Muench (11).

#### EXPERIMENTAL

The initial attempts to transmit yellow fever virus with *Haemagogus splendens* were made in saimiri monkeys. Although data were obtained indicating that the mosquitoes probably transmitted the virus, results were not decisive. Therefore, when Bates and Roca (7) described their experiments with the douroucouli, we decided to use that species in view of its more desirable characteristics.

#### TRANSMISSION EXPERIMENTS

Douroucouli No. 3527 was given 333 fifty per cent mouse mortality doses of Chichimene virus subcutaneously. On the fourth day after inoculation, when the monkey had fever, a group of 39 *Haemagogus splendens* were allowed to feed. Serum obtained at the time of feeding had a virus titer of  $1:4.0 \times 10^7$ . The following day, another group of 31 mosquitoes was fed and the serum at that time was found to have a virus titer of  $1:2.1 \times 10^8$ . This monkey died on the sixth day after inoculation.

The mosquitoes were kept in a constant temperature incubator at 30°C. until

<sup>1</sup> The authors wish to express their gratitude to Dr. Marston Bates who kindly supplied them with many of the monkeys used in these experiments.

they were used. Since the serum virus titer of the source monkey was very high, it was decided to make the first attempt to transmit after a 9-day incubation period. Three mosquitoes were, therefore, allowed to bite douroucouli No. 3528. After engorging, the mosquitoes were individually triturated and inoculated into groups of baby mice; two of the three were found to contain virus. The monkey showed no evidence of infection, and serum taken on the fifth and seventh days after exposure failed to infect mice. A serum sample obtained 30 days after exposure gave a negative result in the protection test.

After a 14-day incubation period, another group of three mosquitoes was allowed to bite douroucouli No. 3539. This monkey likewise failed to show evidence of clinical infection or circulating virus, although each mosquito was found to contain virus. Again, the serum sample taken 30 days after exposure did not contain specific antibody.

Douroucouli No. 3540 was bitten by five *Haemagogus splendens* that had been kept for 19 days. Four of the five mosquitoes were found to be infected, a specificity test indicating the virus to be that of yellow fever. This monkey became ill on the fifth day after exposure. The disease progressed very rapidly, and the animal died the same day. Serum inoculated intracerebrally into white mice showed the presence of yellow fever virus, as proved by a specificity test. The titer of virus in the serum was in excess of  $1:1 \times 10^5$ , the highest dilution tested.

Douroucouli No. 3541 was bitten by five mosquitoes that had also been kept for 19 days. All of them contained virus. These mosquitoes had fed on douroucouli No. 3527 during the fifth day of its infection, whereas those feeding on No. 3540 had been fed on No. 3527 on the fourth day of infection. Monkey No. 3541 became ill four days after exposure and died later that day. However, before its death, a group of 18 normal *Haemagogus splendens* engorged on it. The serum, at the time of feeding, had a virus titer of no less than  $1:1 \times 10^8$ , the end point not being reached. The virus isolated proved to be that of yellow fever, as indicated by the specificity test.

In order to ensure cyclic transmission, another douroucouli, No. 3543, was infected by bite of a group of five *Haemagogus splendens* that had been kept for 22 days after taking their infectious meal. Again all of the mosquitoes were found to be infected. This animal, although not clinically ill, was used to feed 30 normal *Haemagogus splendens* on the fourth day after exposure. The serum had a virus titer of no less than  $1:1 \times 10^8$ , the end point again not being reached. The following day another group of 44 normal mosquitoes was fed, and the serum at that time had a titer of  $1:2.5 \times 10^7$ . This monkey died on the sixth day after being bitten.

It was thought that these three lots of infected mosquitoes might be used to define the incubation period with a little more precision as well as to confirm the first transmission. In the first experiment, transmission was obtained after 19 days but not after 14 days.

Mosquitoes that fed on douroucouli No. 3541 were allowed to bite douroucouli No. 3534 after a 16-day interval. The monkey became ill and died on the fourth



day after exposure. Virus isolated from serum obtained before death was shown by specificity test to be yellow fever virus.

Four more of this group of mosquitoes were allowed to bite douroucoulis No. 3542 after an 18-day incubation period. This monkey died on the fifth day after exposure, and yellow fever virus was isolated from the blood, also proved by specificity test. All of the mosquitoes used to infect the last two monkeys were found to contain virus.

Transmission  
cycle.

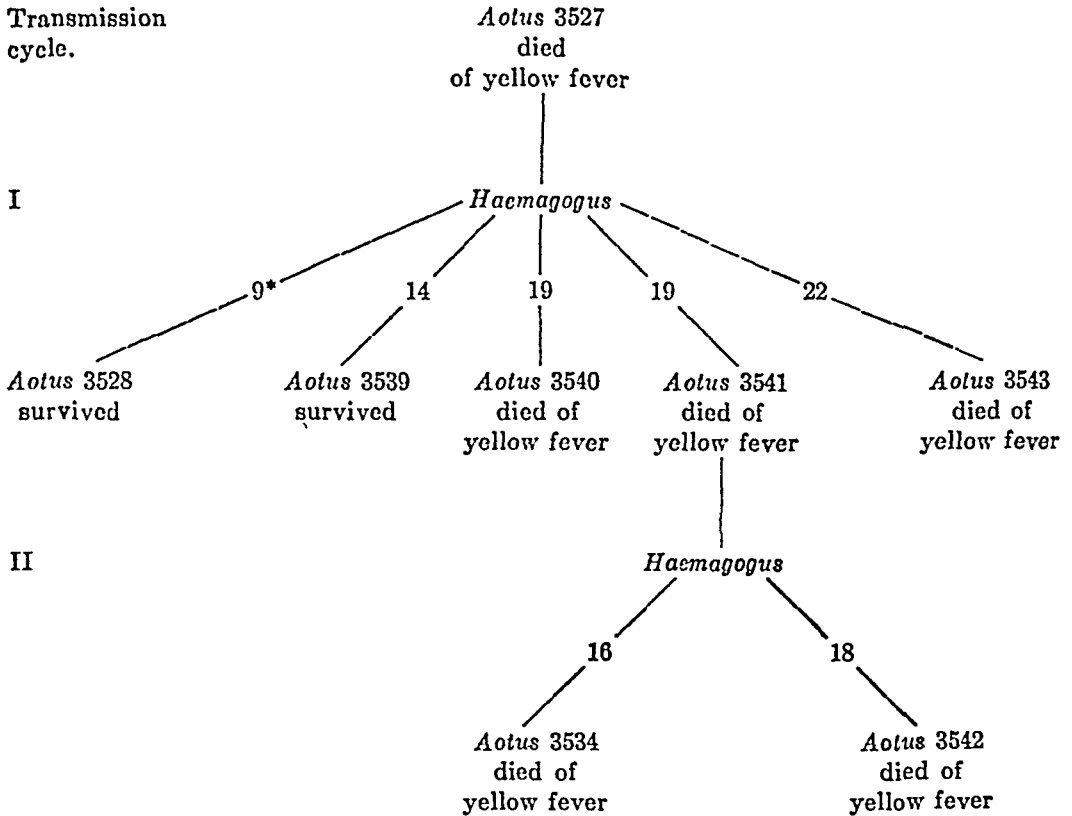


FIG. 1. DIAGRAM OF CYCLES WITH HAEMAGOGUS SPLENDENS

\*The figures represent the incubation period in days at 30°C.

The data have been summarized graphically in Figure 1.

All of the monkeys that died of yellow fever were found to have lesions in the liver characterized by a coagulative necrosis similar to that observed by Gast in histological examination of liver material from douroucoulis used in the infection experiments of Bates and Roca (see (7), Bates and Roca-García, 1945).

It can therefore be seen that *Haemagogus splendens* is a proved laboratory vector of yellow fever virus and a potential natural vector. The "extrinsic incubation period" has been found to be between 14-16 days in mosquitoes kept at constant temperature of 30°C. This is very close to the interval found necessary to obtain transmission with *Haemagogus spegazzinii falco* by Bates and Roca

(5). Proof of the rôle of *Haemagogus splendens* in the epidemiology of jungle yellow fever awaits the evidence that can be supplied only by field studies.

#### RELATIONSHIP OF VIRUS TITER IN THE MONKEY-TO-MOSQUITO INFECTION

Bates and Roca (5) have discussed several of the factors concerned in the infection of *Haemagogus spegazzinii falco* with yellow fever virus. One of these factors was found to be the amount of virus ingested. This is, naturally, related to the titer of virus in the circulation of the source animal. During the course of these experiments, a number of mosquitoes were tested for virus, after a 10-day incubation period, following engorgement on animals with widely varying virus

TABLE I

*Influence of virus titer on the percentage of mosquitoes found infected after a 10-day incubation period*

ANIMAL USED AS INFECTIOUS MEAL	VIRUS TITER	TEMPERATURE AT WHICH MOSQUITOES WERE KEPT	NUMBER OF MOSQUITOES TESTED	PERCENTAGE OF MOSQUITOES INFECTED
(1) Saimiri	$1:3.2 \times 10^2$	30°C.	75	0
	$1:1.3 \times 10^3$	30°C.	45	2
(2) Saimiri	$1:1.4 \times 10^3$	25°C.	6	0
(3) Saimiri	$1:3.0 \times 10^4$	30°C.	9	22
	$1:2.0 \times 10^3$	30°C.	28	0
(4) Saimiri	$1:1.7 \times 10^4$	30°C.	39	5
(5) Saimiri	$1:1.6 \times 10^3$	30°C.	24	100
(6) Aotus	$1:4.3 \times 10^7$	30°C.	7	86
	$1:2.1 \times 10^8$	30°C.	10	100
(7) Aotus	$1:1.0 \times 10^{8*}$	30°C.	9	100

\* The end point of the titration was not obtained.

titers. The presence of virus was determined by inoculating intracerebrally into mice a suspension of the triturated mosquitoes. The results are presented in Table I. It can be seen that with the higher titers almost all of the mosquitoes are infected, and that as the titer diminishes the percentage of virus-containing mosquitoes likewise diminishes. Titers of about 1:1,000 seem to be critical, no virus being recovered from mosquitoes feeding on animals with a low titer

#### SUMMARY

It has been demonstrated that *Haemagogus splendens* will transmit yellow fever in the laboratory. The extrinsic incubation in this species was found to be between 14 and 16 days in mosquitoes kept at a constant temperature of 30°C.

The titer of circulating virus of the source animal was found to be related to the percentage of mosquitoes infected.

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# THE DISTRIBUTION OF MURINE TYPHUS IN RATS AND IN HUMANS IN SAN ANTONIO

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## INTRODUCTION

Murine typhus fever, because it is primarily a rat disease, will naturally occur with greatest frequency in humans where the rat population is the highest. However, the mere presence of rats does not mean that typhus is also present. Other factors, especially the number of fleas and the density of rats in an area, must be considered as well as the presence of the typhus agent.

The aim of this paper is to describe the widespread distribution of typhus in rats in various areas of the city of San Antonio and to show the origin of human cases. Previous investigations of murine typhus fever (1, 2) have emphasized the localized distribution of the disease in the commercial districts and in connection with food handling establishments especially.

The prevalence of typhus in rats was determined by the presence of antibodies as indicated by the complement-fixation test for murine typhus. The human cases were reported to the San Antonio Health Department and were then investigated to determine the most probable source of infection. A special effort was made in 1944 to improve reporting by interviews with physicians. In 1945 for the typhus season an epidemiologist was assigned to the Health Department. He improved reporting by personal interviews, by letters, and through the cooperation of the Bexar County Medical Society. In most instances rats were trapped at one or more supposed sources to determine if complement fixing antibodies or the typhus agents were present.

San Antonio has about an equal number of the two species of commensal rats. The Roof Rat (*Rattus rattus*) is most common in the downtown business district and in houses and garages. The Brown Rat (*Rattus norvegicus*) is most common in grain mills, grocery stores and around chicken coops. In some places both species occur in the same building. The most common flea is the rat flea (*Xenopsylla cheopis*) which reaches its seasonal peak of abundance in May and June. Another flea (*Leptopsylla segnis*) is most common in March. *Nosopsyllus fasciatus* is rare. The mite *Liponyssus bacoti* is common in winter and *Eulaelaps* sp. (*hawaiiensis*?) is found in the summer.

For the purpose of this work the city has been arbitrarily divided up into areas in relation to typhus fever. These districts are purely artificial and are based on a knowledge of the type of inhabitants and the rat infestation. The boundary lines are arbitrarily drawn and probably could be better arranged in some small sections, but extreme refinement is not possible or necessary. The districts indicated in the map are described in table 1.

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District 1 is the best residential area and has few stores. Districts 2, 3, and 4 are primarily residential but have many corner groceries and some factories and grain mills. Certain parts have poor sanitary conditions. District 6 includes the downtown business center and nearby factories and warehouses. There are many residences, some good, some poor, scattered throughout the districts. District 5 stands apart from the others because of its poverty, inadequate sanitary conditions and the nearby produce markets.

TABLE 1  
*Characteristics of the districts of San Antonio*

DISTRICTS	TYPE	RACE	PERSONS PER ACRE	STORES	SLUMS	GRAIN MILLS	RATS
1	Fine residential	Whites	10-15	Good shopping centers	None	None	Rare
2	Business, residential	Whites, few Negroes	15-25	Small factories, many corner groceries, etc.	Few blocks	Several	Common
3	Fair and good residential	Whites, Negroes	10-20	Corner grocery, poor shopping centers	Certain parts	None	Fairly common
4	Fair and good residential	Whites, Mexicans, few Negroes	25-30	Many corner groceries, poor shopping centers	Certain parts	Few	Common
5	Slum, residential, business	Mexicans	45-80	Factories, poor stores	Complete	Several	Abundant
6	Business	Whites	10-15	Commercial district	None	None	Abundant

#### THE DISTRIBUTION OF TYPHUS IN RATS

The distribution of typhus in rats (table 2) has been determined by the complement fixation test, which indicates that the rats have been infected in the past. In a few cases the typhus agent was isolated from the brain and in several cases the flea feces were tested for rickettsiae. The rats are divided into species and totaled according to the type of establishment. Young rats are individuals not yet sexually mature. Young Roof Rats usually measure less than 140 mm. for length of body plus head, and young Brown Rats usually measure less than 160 mm.

The rats tested in districts 3, 4, 5, and 6 are a random sample of the rats which were collected by going from block to block looking for suitable places to trap

rats. The figures include, however, rats trapped at the suspected sources of typhus and hence are slightly biased. The rats from districts 1 and 2 are biased because systematic trapping was not done except in a few shopping centers. About half of these rats came from suspected sources of typhus.

Evidence of the presence of typhus fever has been found in all districts. Districts 1 and 3 have definitely low percentages of positive rats. The small numbers of rats tested reflects the fact that these districts have few rats which are found in only a few widely-separated stores or chicken coops.

TABLE 2  
*Percentage of positive rats by districts*

	DISTRICT 1		DISTRICT 2		DISTRICT 3		DISTRICT 4		DISTRICT 5		DISTRICT 6	
	Rats	Per cent	Rats	Per cent	Rats	Per cent	Rats	Per cent	Rats	Per cent	Rats	Per cent
<i>Rattus rattus</i>												
Adults.....	16	13	26	31	12	8	46	11	147	52	35	31
Residences.....	8	25	2	50	7	0	36	14	39	49	0	
Stores.....	8	0	4	50	5	20	10	0	38	42	35	31
Mills.....	0		19	26	0		0		60	53	0	
Young.....	12	17	32	13	15	13	68	7	108	21	51	14
Residences.....	3	67	14	14	3	33	56	7	31	13	0	
Stores.....	9	0	3	15	14	7	12	7	28	71	51	14
Mills.....	0		15	0	0		0		49	35	0	
<i>Rattus norvegicus</i>												
Adults.....	31	16	98	61	17	35	41	22	126	66	62	50
Residences.....	10	40	9	56	10	40	27	9	46	63	0	
Stores.....	21	5	16	69	7	29	9	5	42	59	62	50
Mills.....	0		73	60	0		5	80	38	76	0	
Young.....	14	36	57	35	14	21	29	7	86	43	31	45
Residences.....	9	56	14	57	5	40	21	5	49	30	0	
Stores.....	5	0	12	50	9	11	3	0	23	9	31	45
Mills.....	0		7	20	0		5	20	14	64	0	

Districts 2, 4, and 6 may be put in one group according to the percentages of positive rats although the districts are not similar in other characteristics. The presence of typhus in these areas is usually dependent upon local groups of stores or poor dwellings with heavy infestations of rats and some parts of these districts are free of rats and typhus. The downtown business area, because of the large number of food establishments, has many rats.

District 5 stands apart from all others because of the high percentage of positive rats. It consists of slums inhabited by very poor Latin-Americans and having poor cafes and small groceries. In addition there are many produce stores and grain mills. The large number of rats tested reflects the fact that rats are abundant and that much trapping was done in the district because of the amount of typhus.

## THE OCCURRENCE OF HUMAN CASES (MAY 1944-OCT. 1945)

The number of cases in each district is summarized in table 3. The column "source" means that the case most probably originated in the district. Under the column "unknown" are noted the residences of cases of unknown origin. Frequently it was certain that such a case was contracted within the district but the exact place was unknown.

The most probable origins of the cases are located on the map. Cases of unknown origin are not located but are totaled in table 3.

Control measures consisting of rat-proofing and eradication were begun in the downtown area (district 6) in August 1944 and probably prevented some cases there in 1945. A control program of killing fleas by dusting rat runs with DDT was conducted in districts 4, 5, and part of 3 in the Spring of 1945 and

TABLE 3  
*Probable source of typhus in districts (May 1944-Oct. 1945)*

DISTRICT	YEAR	SOURCE IN DISTRICT	SOURCE UNKNOWN RESIDENCE IN DISTRICT
1	1944		9
	1945	6	2
2	1944	10	6
	1945	26	9
3	1944	13	14
	1945	8	6
4	1944	2	2
	1945	6	2
5	1944	13	2
	1945	10	4
6	1944	8	1
	1945	3	

apparently prevented the occurrence of some typhus cases in those districts. Hence the map and table 3 would probably have shown more cases in these two areas.

The map and table 3 show the widespread occurrence of typhus throughout the city. Positive rats and human infections occur in all districts although there are considerable differences among districts.

The occurrence of typhus fever in district 1 in 1945 is possibly an example of extension of distribution of infected rats. In 1944 no human case was traced to that district although several persons who contracted typhus lived there. A survey of the shopping centers indicated few rats and all of 16 rats tested were found to be negative. In 1945, 6 cases were clearly contracted in district 1 and positive rats were caught at 4 places, one being a store in which only nega-

tive rats were found in 1944. Unfortunately the rat-survey in 1944, due to scarcity of rats, was inadequate to prove the absence of positive rats, but the

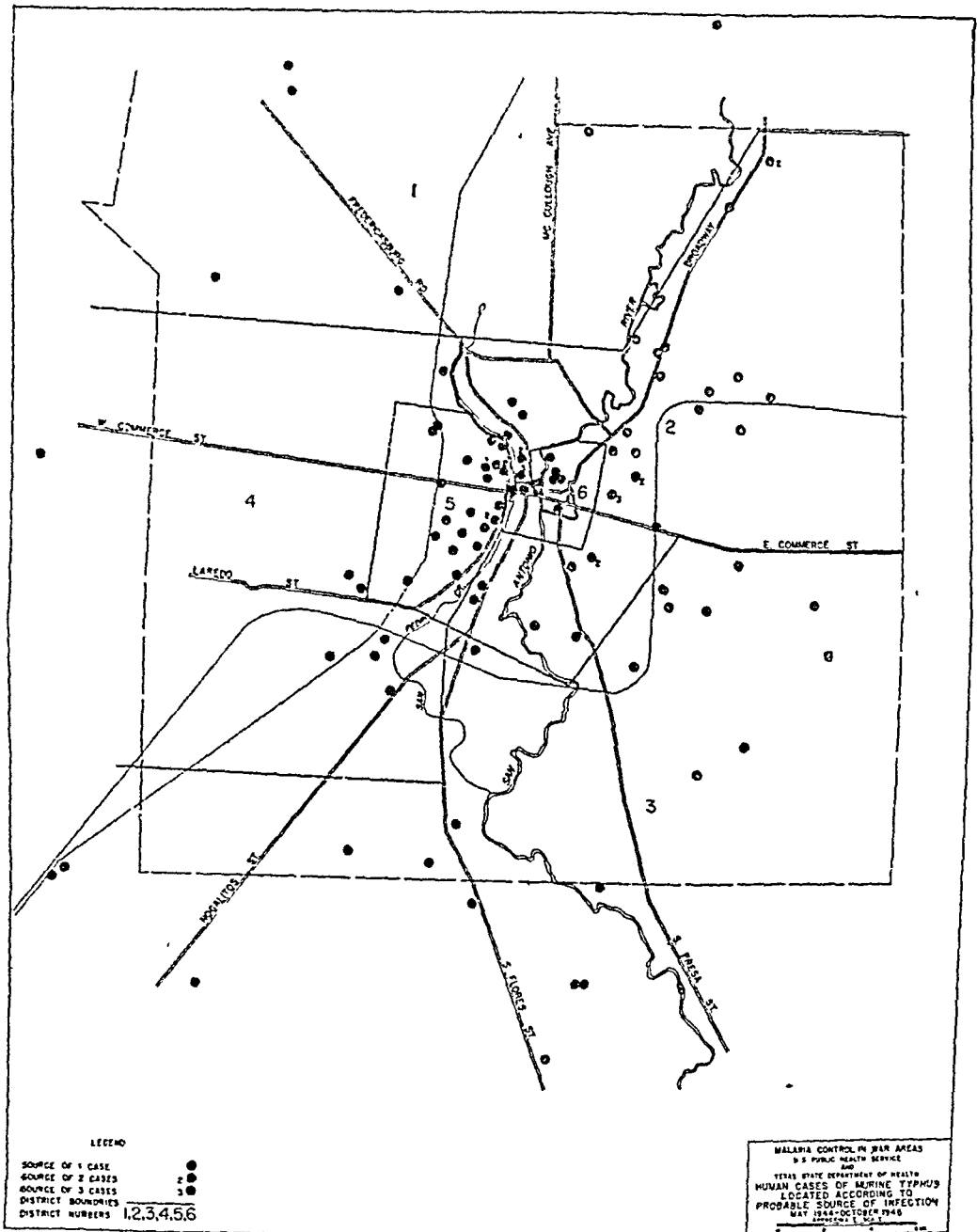


FIG. 1

available evidence does suggest that typhus did invade the good residential district in 1945. However there is a possibility that a certain woman became infected in district 1 in 1943.



The number of cases of unknown source but living in districts 2, 3, and 5 is noteworthy. In many cases it was obvious that the person became infected near the residence. In contrast district 1 is very different. In 1944 the cases of unknown source living in district 1 appear to have become infected elsewhere. Seven of the 9 persons were men; 1 was a retired colonel and 8 worked downtown; one of these owned a restaurant and another was manager of a wholesale grocery. There is no evidence that anyone became infected in district 1 in 1944.

There is only a general correlation between percentages of positive rats and the number of human cases in a district. The districts with high percentages of positive rats tend to have most typhus cases. But, for example, district 3 has many cases of typhus but not a high percentage of positive rats. Typhus in that district depends upon small centers of poor houses or stores and upon rats at chicken coops. The occurrence of typhus in other districts supports the idea that infection in rats is frequently localized in small areas. The methods designed to control typhus fever, therefore, should be aimed at small localized sources of infection instead of at general districts.

#### SUMMARY

To determine the spatial distribution of murine typhus fever in San Antonio in 1944 and 1945 rats were collected throughout the city and tested for the presence of complement fixing antibodies for typhus. The human cases were investigated to determine the most probable source of infection. The city was divided into 6 arbitrary districts according to the characteristics of the inhabitants and amount of typhus.

Rats positive for typhus fever have been found in all districts but the good residential areas have low percentages of positives. The fairly good residential areas and the business districts have about 40 per cent of rats positive. The very poor slum district has about 60 per cent of the rats positive.

Human cases originated in all districts and showed a general but not detailed correlation with the percentages of positive rats. Apparently human infections depend upon a local concentration of positive rats rather than a uniform distribution of infected rats in an area.

#### ACKNOWLEDGMENTS

This study was made possible by the cooperation of the City Health Officer, Dr. Lewis C. Robbins, and his staff. Dr. E. R. Rickard assisted in confirmation of diagnoses and investigation of human cases. Col. Charles F. Craig, U. S. Army, Ret., Chairman of the San Antonio Typhus Advisory Committee, gave advice freely during the work.

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# PATHOLOGIC STUDY OF NATURAL AMEBIC INFECTION IN MACAQUES<sup>1, 2</sup>

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In 1904, Musgrave and Clegg reported that *Macacus cynomologus* and *M. philippinensis* naturally contracted amebiasis and developed ulceration of the colon (1). This evidence, and that derived from Kessel's investigation as well, favored the conclusion that the intestinal protozoa of macaques and man are identical. Six macaques were autopsied by Kessel, 4 had no mucosal changes. In 2 animals amebas were found in the mucosa and submucosa and also in the lymph follicles.

*Endamoeba histolytica* then, naturally infecting monkeys, is believed to be identical with the protozoan which causes amebiasis in man. This opinion is based on the studies of morphology by Kessel (1), Hegner and Chu (2), and on the work of Dobell (3), and Hegner et al. (4), who were able to infect *Macacus rhesus* with pathogenic amebas isolated from man. Craig and Swartzwelder (5), observed that monkeys, naturally or experimentally infected with amebas, gave positive complement fixation tests.

Because of the similarity between amebic infection in man and macaques, naturally infected monkeys were used in the testing of potential amebacides by Anderson and Koch (6).

The pathologic data presented in this study tend to support the reports of Kessel (1) and Hegner et al. (4), and Johnson (7), who have shown that *E. histolytica* is a tissue parasite of monkeys. A careful gross and microscopic examination was made of 5 monkeys that died or were sacrificed during unsuccessful treatment with possible amebacides. Tissues for the histopathologic study were fixed in Schaudinn's solution and stained with hematoxylin and eosin and Heidenhain's iron hematoxylin solutions.

## NATURE OF THE INFECTION

Though none of the monkeys showed symptoms of dysentery, the presence of amebas in the 5 animals was established by microscopic examination of the stools. These were fixed in Schaudinn's solution and stained with iron hematoxylin (8). Cysts of *E. histolytica* were found in about 75 per cent of the stools examined. Other amebas likely to be confused with *E. histolytica* were also

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research, Office of Scientific Research and Development, and the University of California. With the technical assistance of Elsa M. Zitcer.

<sup>2</sup> Read at the Annual Meeting of the American Society of Tropical Medicine, at Cincinnati, Ohio, November 14, 1945.

noted: viz, *Endamoeba coli*, *Endolimax nana* and *Iodamoeba butschlii*. Kessel (1), Dobell (9), and Hegner and Chu (2), have shown that these are the same species as found in man. Blastocysts and flagellates were also found.

*E. histolytica* occurred in the form of both trophozoites and cysts. Quadri-nucleated cysts, found at intervals, were considered pathognomonic for infection. Usually, however, the cysts were uninucleated. They were never numerous, rarely more than 3 in a stained smear, and were greatly exceeded in number by the cysts of *E. coli*. A similar finding was reported by Lincicome (10), who counted the cysts passed during 24-hour periods.

The forms of *E. histolytica* showed considerable variation in size, and appeared to fall into two classes. In the smaller race, the trophozoites and the uni- and quadrinucleated cysts were about  $5\mu$  in diameter. In the larger race, the trophozoites were from  $13\mu$  to  $17\mu$ , the precysts from  $6\mu$  to  $10\mu$ , and the uni- and quadrinucleated cysts were from  $6\mu$  to  $14\mu$  in diameter. These measurements are smaller than those reported by Hegner and Chu (2).

*E. coli* also was generally smaller than reported by Hegner and Chu (2). The trophozoites ranged from  $8\mu$  to  $22\mu$ , the binucleated cysts from  $9\mu$  to  $14\mu$  and the octanucleated cysts from  $12\mu$  to  $18\mu$  in diameter. Measurements were not made of other species.

#### COURSE OF INFECTION

Of the 5 animals selected for study, one had not been treated. One had been treated and subsequently passed no cysts in the 58 days following therapy. A few cysts were found in stained smears made from the contents of the bowel at the time of autopsy. This animal had passed numerous cysts before treatment. The other 3 animals had been unsuccessfully treated several times. During the period of therapy all amebas disappeared from the stools. Immediately following treatment the non-pathogenic species were again found. *E. histolytica* was absent from the stools for from 3 to 8 days after treatment and then reappeared. One of these monkeys died after another unsuccessful treatment. Another died during treatment and the third died after treatment with emetine. In the latter animal amebas could not be found in stained smears of fecal material, however culture from the contents of the bowel was positive for *E. histolytica*.

#### GROSS PATHOLOGIC FINDINGS

All animals were completely studied at autopsy, except for the head. In the intestinal tract, abnormalities were limited to the large bowel. There was some generalized congestion and in a few areas in each animal, except one, scattered pin-point mucosal hemorrhages were seen. These appeared to be very superficial and not limited to any one portion of the large bowel. No gross evidence of ulceration was found. The other tissues examined were not unusual except for the presence of tuberculosis.

#### MICROSCOPIC DESCRIPTION

The pictures presented by sections of the large bowel were essentially similar throughout in all 5 monkeys. A patchy, low-grade, chronic inflammatory re-

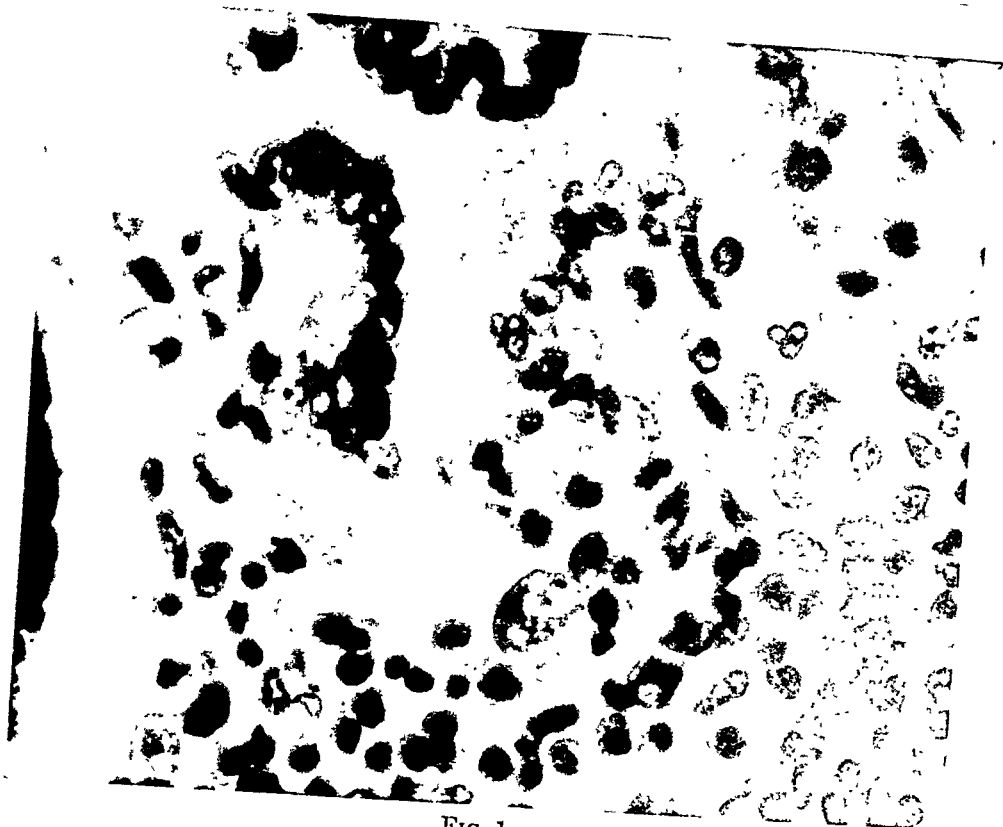


FIG. 1

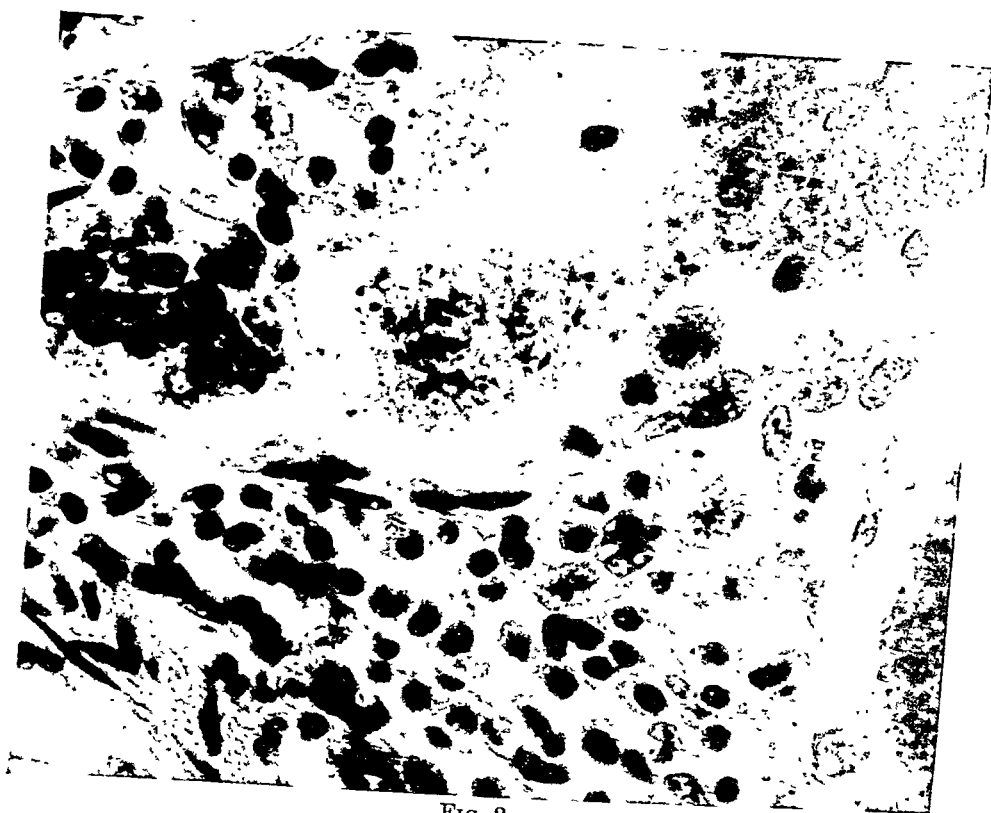


FIG. 2

FIGS. 1 AND 2.  $\times 800$ . LARGE INTESTINE SHOWING AMEBAS LYING IN THE SUPERFICIAL MUCOSA

In figure 1 the ameba lies among the lamina propria cells, and in a small lumen of apparent tissue lysis. In figure 2 the organism is shown progressing along the interglandular stromae papilla.

action of the mucosal layers was observed. This was characterized by edema, increased numbers of lymphocytes and plasma cells together with varying numbers of macrophages often showing phagocytosis. Foci of interstitial hemorrhages were encountered occasionally, and rather rarely a shallow ulcer in the mucosa was noted. More frequently there were seen cystic solitary glands of Lieberkühn that were dilated to 3 or 4 times their usual size. These contained serous material, cellular debris and scattered polynuclear leucocytes. There was little surrounding inflammatory reaction.

No amebas were found in or around the superficial ulcers. However, in at least one instance an ameba was intimately associated with an inflammatory, cystic, rectal gland. All amebas encountered had invaded only as far as the mucosal layer. Many of these lay only in the gland crypts. However, an occasional ameba was seen lying in and invading the tissues (fig. 1). From their position and distribution it seemed probable that they first entered near the mouth of the crypt and then invaded the inter-glandular papillae of the lamina propria (fig. 2). Usually the tissue reaction around these amebas was minimal but in one instance there was a cystic inflammatory Lieberkühn crypt associated with an ameba.

The muscularis mucosa was intact and in the submucosa no abnormalities were seen. The lymphoid tissues presented no amebas; they were not inflamed and showed no "follicular abscess" formation. The muscular and serosal layers were not unusual. In no section was there seen any reaction to tuberculosis.

The amebas seen in the section could usually be identified as to species. Within the lumen of the bowel, *E. coli* were often seen as well as *E. histolytica*. In the tissues the species was *E. histolytica*. In both sites occasional organisms could not be specifically identified.

Sections of the other tissues of these animals revealed no abnormalities except tuberculosis.

#### DISCUSSION

*E. histolytica* was demonstrated invading the tissues of naturally infected macaques. The possibility of this having been a post mortem migration (11) can be ruled out since amebas were found in fresh material fixed immediately after death. Although some gross and microscopic hemorrhages and inflammatory lesions were noted, it is difficult to relate them positively to the presence of amebas. In most instances there were no organisms demonstrated in or around the inflammatory foci. However, since in one animal an ameba was found intimately associated with a cystic and inflamed crypt of Lieberkühn, it cannot be denied that the ameba may have been etiologically related to these lesions as well as to the occasional interstitial hemorrhages, superficial ulcerations and chronic reaction encountered.

The invasiveness of amebas was probably of a low order in view of their limitation to the mucosa. No abnormalities were seen in the lymphoid tissues peripheral to the muscularis mucosa. Such a pathologic finding is consistent with the clinical appearance of the animals. They had no diarrhea, gross

intestinal hemorrhage nor evidence of active amebic disease. Clinically they would have to be considered as cyst passers in a "carrier" phase of the disease, therefore virulent amebic tissue invasion, obvious amebic intestinal lesions and vigorous host reaction could not be expected.

#### SUMMARY

*E. histolytica* were found in the mucous membrane of naturally infected macaques. In all animals examined there were patchy, low-grade, chronic inflammatory reactions and scattered cystic and inflamed crypts of Lieberkühn. The direct relationship of these abnormalities to the presence of the ameba was not positively established, although certainly there seemed to be an invasiveness of a low order. Usually the amebas lying in the tissues were associated with minimal tissue reaction.

The amebas were located only in the mucous membrane layer and from their position would tend to support the idea that they first entered the tissues near the mouth of the cryptal glands where they passed directly into the interglandular papilla of the lamina propria.

On the basis of these pathologic findings, it is our belief that naturally infected monkeys (*Macacus rhesus*) serve as suitable laboratory animals in the study of proposed amebicides.

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# ASIATIC CHOLERA

## CLINICAL STUDY AND EXPERIMENTAL THERAPY WITH STREPTOMYCIN<sup>1</sup>

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After the epidemic of 1873, cholera ceased to be a problem in the United States. Occasional outbreaks occurred on ships arriving from epidemic areas, but the disease never gained further foothold here and perhaps never will except for some unforeseen catastrophe. Cholera is still endemic and periodically epidemic in China, India and elsewhere in the orient, and will continue to be as long as there are poverty and overcrowding. During the early summer of 1945 cholera was an added threat to the military operations in progress in southern China.

In May, much earlier than usual, cholera broke out and soon reached alarming proportions in the overcrowded city of Chungking. In June 839 cases with 132 deaths were reported and in July 1250 cases with 220 deaths. By mid-July the epidemic reached its peak, declined gradually and was over by the end of August. A total of 2374 cases, 392 deaths and a mortality rate of 16 per cent were reported. The statistics underestimate the extent of the epidemic for, without doubt, many severe attacks and deaths and many mild cases were not officially registered because of administrative and other difficulties. Patients with mild attacks seldom seek medical aid and mild attacks cannot be diagnosed as cholera without bacteriologic study. Several ambulatory patients were observed during the epidemic who had only a day or two of malaise, anorexia, vomiting and a few copious watery stools containing *V. comma*. So far as is known only 6 or 7 occidentals contracted cholera during the epidemic.

To combat the epidemic, local Chinese health officers set up 720 beds in 10 emergency cholera hospitals established in early July in different parts of the city. The hospital where these studies were made was hastily organized in a week and housed in an abandoned theater large enough to hold 72 bamboo pallets. A partition of matting down the center divided the beds equally for men on one side, women and children on the other (fig. 1). The floor was earthen and additional windows were knocked in the walls. Windows and doors were screened with cloth mesh. An adjacent screened building contained an office, sleeping and dining quarters for the staff doctors and a laboratory with meager equipment for counting blood, staining slides, and preparing fluids for intravenous injection.

<sup>1</sup> The streptomycin used in this study was generously supplied by Merck & Co., Inc., Rahway, N. J.

<sup>2</sup> Medical Officer for the study of cholera, United Nations Relief and Rehabilitation Administration, Chungking; and from the Jefferson Medical College and Hospital, Philadelphia, Pa.



The staff consisted of a director, five other physicians, and a variable number of nurses, aides and coolies depending upon the number of patients present. All were vaccinated against cholera and instructed in special nursing and antiseptic technic. None contracted the disease.

During the existence of the hospital from July 12 to August 31, one hundred and sixty patients were treated. Of these about 100 had clinically typical cholera with bacteriologic proof; 40 were clinically typical cholera without bacteriologic proof, and 20 probably had other disease. Later in the study, improved technic, as described later, gave a higher percentage of positive cultures.



FIG. 1 SECTION OF AN EMERGENCY CHOLERA HOSPITAL IN A CINEMA THEATER  
Beds are arranged in 3 rows—A, B and C of 12 beds each and numbered accordingly. Women's and children's section is on the other side of the partition at the left.

#### GENERAL MANAGEMENT

After admission of a patient to the hospital by way of stretcher, rickshaw, cart, or on the back of a friend, a history and physical examination were recorded. Two glass tubes were in turn inserted into the rectum, a specimen of stool was obtained and examined by a smear stained with dilute fuchsin, and the rest was sent to the laboratory at the Central Hospital for culture and identification. A blood count was made and the specific gravity of the blood was measured at the bedside with the copper sulfate solution droplet suspension technic (1).

The patient was then placed on a pallet made of strips of bamboo in a frame supported by two trellises, and in which a hole 6 in. x 6 in. was cut near the center for the passage of stools in the recumbent or sitting position (fig. 2). The stools discharged directly into a 1 per cent solution of cresol in a pot held by a pedestal. At the bedside, a basin for vomitus contained the same solution. Urine was



FIG. 2. CHOLERA PATIENT ON A FENESTRATED PALLET, SHOWING POT FOR THE COLLECTION OF STOOLS AND A BOWL FOR VOMITUS IN PLACE  
A spike-tipped staff driven into the earth supports the infusion apparatus



FIG. 3. METHOD OF COLLECTING EXCRETA AT THE BEDSIDE AFTER MEASUREMENT  
The basin at the left is handled with tongs, and the pot with hooks

collected separately. All dejecta were measured and recorded daily. The receptacles were emptied into a bucket on a cart wheeled to the bedside by an attendant who used iron hooks or tongs to avoid soiling his hands (fig. 3). Spill-

age was covered with lime and sprayed with cresol solution. The bucket of collected excrement was emptied into a deep covered pit outside.

Since cholera must be regarded as a therapeutic emergency, intravenous infusion of 1000 cc. to 2000 cc. of warmed physiologic salt solution was given immediately, the amount determined by the size of the patient and the degree of dehydration as measured by the specific gravity of the blood. About 300 cc. of 2 per cent sodium bicarbonate solution was added to each injection to combat acidosis when collapse was present or threatened. The flow was adjusted from 60 cc. to 100 cc. a minute. Infusions were given by the intrasternal route to great advantage in 8 patients whose veins were inaccessible. The specific gravity of the blood was measured before and after each injection, and if high afterward, more fluid was given until the normal of 1.054 to 1.058 was attained. The patient was given 50 cc. or more of 1 per cent solution of sodium bicarbonate to drink every 15 minutes during the acute stage. Patients were urged to drink water or tea and were given liquid, then solid food as soon as they desired it. Chills were relieved by hot water bottles and padded quilts.

Amazing improvement usually came during or after rehydration, with return to consciousness; recovery from collapse, hypotension, hypothermia, purging, vomiting and cyanosis; cessation of cramps; an increase of urine and filling out of the shrunken skin. In some patients, continued loss of fluid and repeated collapses required repeated infusions, each controlled by specific gravity measurements of the blood. Fluid loss in case 3 amounted to 7300 cc., nearly two gallons in one day. Smears and cultures of the stools were made daily or every second or third day. Patients were kept in the hospital seven to ten days but often insisted upon leaving sooner. The stools were usually vibrio-free by this time. Under these favorable conditions for treatment only 7 cholera patients died or 5 per cent. The low mortality rate was ascribed to prompt, vigorous, controlled rehydration and the restoration of mineral metabolites, repeated when necessary, and good medical care by a relatively large, well-trained staff. The average duration of the disease, thus treated, was about 4 days. It was difficult at times to date the end-point of the attacks; recovery was recorded at the time the diarrhea stopped and the patient felt well. In some instances diarrhea persisted a day or two after symptoms ceased; in some, diarrhea stopped first, and in others, both ended at once. Uremia and other severe manifestations which sometimes develop in cholera did not occur except in the few who died. Of these, 3 had severe attacks and were treated late in the disease, two were over 60 years old, one had malaria also, and in one the diagnosis was uncertain.

#### BACTERIOLOGIC METHODS

Cultures at first were made from the passed stools, but the percentage of positive cultures later was increased by obtaining samples from the rectum. Two glass tubes (fig. 4) in turn were inserted about three inches into the rectum, drawn back and forth gently several times until fluid entered the side aperture and collected in the rounded end.

A smear on a glass slide made from material adhering to the outside was stained

and examined, and the tube was placed in a sterile test tube containing alkaline peptone water at pH 8.4 (2). The other specimen tube was placed in a test tube containing boric acid-sodium chloride preservative medium at pH 9.2 (3), a procedure of especial value. Both tubes were sent to the laboratory immediately. The peptone water tube was placed at 37°C. for six to eight hours and the inoculated preservative medium was kept for later study if needed.

At the end of six to eight hours the peptone water culture was observed for turbidity and a surface pellicle. A smear was made and stained by Gram's method and dilute carbol fuchsin. If organisms typical of *V. comma* were seen, a hanging drop preparation was made to observe their motility. From the same culture, a slide agglutination test was made using immune cholera O-group 1 serum, diluted 1 to 100, together with a control preparation. Agglutination gave presumptive identification which was confirmed by further study.

In the case of scanty growth or of doubtful identity, subculture was made in another tube of peptone water and the above described procedure was repeated.

From either the first or second peptone culture, which may contain a variety of bacteria, several loopfuls of the surface growth were streaked on alkaline

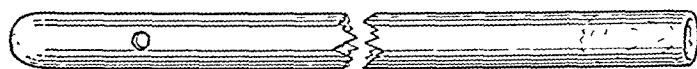


FIG. 4. ONE-QUARTER INCH GLASS TUBE, 7 INCHES LONG WITH A PORE NEAR THE CLOSED END, FOR THE COLLECTION OF LIQUID STOOLS FROM THE RECTUM

(pH 8.4) nutrient agar plates and incubated at 37°C. overnight. Likely colonies of *V. comma* were glistening, translucent, flat domes. A third of a typical colony was used for a stained slide preparation to identify comma forms; if present, one-third of the colony was used for an agglutination test made as described before. If positive, clumping can be seen easily within five minutes. Microscopically, there is entanglement and loss of motility. When this occurs, the remainder of the colony is studied as presently described.

Meanwhile, a portion of the peptone water subculture is used for the cholera-red test but a positive reaction is non-specific and is used only for further support of identification.

When no suspicious colonies were found on the agar plate, the specimen preserved in boric medium was tested. Transfer of 0.1 ml. was made to alkaline peptone water and incubated. If even this procedure failed to reveal *V. comma* it is unlikely that any were present in the specimens and a negative report was given.

The procedures just described identified *V. comma* isolated from most of the cholera patients. In case of doubt with inagglutinable vibrios, other studies were needed for identification, such as fermentation tests with saccharose and arabinose, the standard hemolysis test with goats' cells, detailed serologic examination with serum of O sub-types Ogawa and Inaba, and lastly the Pfeiffer phenomenon in guinea pigs. The bacteriologic procedures are meticulous and time consuming. Strains of *V. comma* isolated during the study were later

classified as of the Ogawa variety by Doctor Seastone at the Army Medical Center in Washington.

#### EXPERIMENTAL STREPTOMYCIN THERAPY

Streptomycin, an antibiotic obtained from *Actinomyces griseus*, inhibits the growth or kills a variety of gram-negative bacilli, including cholera vibrios (4). Some laboratory strains of *V. comma* tested elsewhere (5) showed no growth after 18 hours incubation in concentrations of 2.25 micrograms while others required 5.5 micrograms for bacteriostasis. *V. comma*, therefore, seemed to be more sensitive than *E. typhosa* which is inhibited by 6 to 18 micrograms per cc. of broth and less sensitive than *Past. tularensis* which requires 0.5 micrograms. Vibrios isolated during this study and subsequently tested by Dr. W. F. Elias in Philadelphia varied greatly in their sensitivity to streptomycin as discussed later.

According to previous work (6), streptomycin given orally is nearly all excreted in the feces where it accumulates in amounts far greater than the theoretical level usually needed for bacteriostatic or bactericidal effects. Doses of 1 to 4 grams (1,000,000 to 4,000,000 units) provide from 4000 to 19,000 micrograms per gram of feces. This amount is enough to eliminate most gram negative drug-sensitive bacilli from the stool as long as the level is maintained. Soon after therapy is stopped, streptomycin disappears and the usual flora returns. Only traces of streptomycin appear in the blood and urine after oral administration. When injected parenterally in 4 gram amounts a day, only traces appear in the feces, 5 to 32 micrograms are attained per cc. of serum and from 40 to 70 per cent is excreted in the urine. In certain cholera patients treated parenterally, the amount of streptomycin in the blood probably rose higher during urinary suppression.

With these facts in mind, the effect of streptomycin on cholera was studied. Since cholera seems primarily to be a local enteric disease rather than a systemic infection like typhoid or tularemia, it seemed important to attain a large amount of the drug as quickly as possible in the intestinal tract. Since *V. comma* seldom penetrates tissues or organs, its destruction in the lumen of the tract theoretically should cure the disease. Oral therapy was therefore first tested, bearing in mind the possibility that vibrios may also live in the gall bladder and elsewhere, and require parenteral injection to bring them in contact with the drug.

Unfortunately, the lack of facilities and equipment prevented measurement of the amounts of streptomycin attained in the stools and serum. Since the optimal dosage was unknown, various amounts of the drug and routes of therapy were tested as described in the case reports. Some of the drug was occasionally lost in vomitus but this was largely avoided by rehydrating the patient as needed. Administration by duodenal tube was not attempted. The current dosage could not be regulated by bacteriologic control because the time consuming cultural and serologic procedures delayed reports of the presence or absence of vibrios for five or six days after the collection of cultures.

Only severely sick patients with typical symptoms of cholera and whose rectal smears contained likely cholera vibrios were selected for treatment. All were

Chinese of the poorest class. In almost all cases, streptomycin therapy began after rehydration on the first day of disease. In one instance treatment was begun in a patient with clinically typical cholera in spite of the absence of vibrios in the smear, which happens at times (7). Treatment of patients solely with streptomycin was not risked.

#### CASE REPORTS

*Case 1.* A woman aged 41, was suddenly attacked with copious watery diarrhea and vomiting on August 6. Twelve stools were passed before she came to the hospital eight hours later. There were repeated, painful muscular cramps in her legs, substernal oppression and anuria. She was in a state of collapse, dehydrated and cyanotic. The specific gravity of the blood was 1.066. The stools on the first day measured nearly 3000 cc.; they were colorless, yellowish, watery, contained white flakes and gushed out painlessly, without effort. Many vibrios were present in a stained smear (fig. 5) and *V. comma* was identified by culture and specific agglutination.

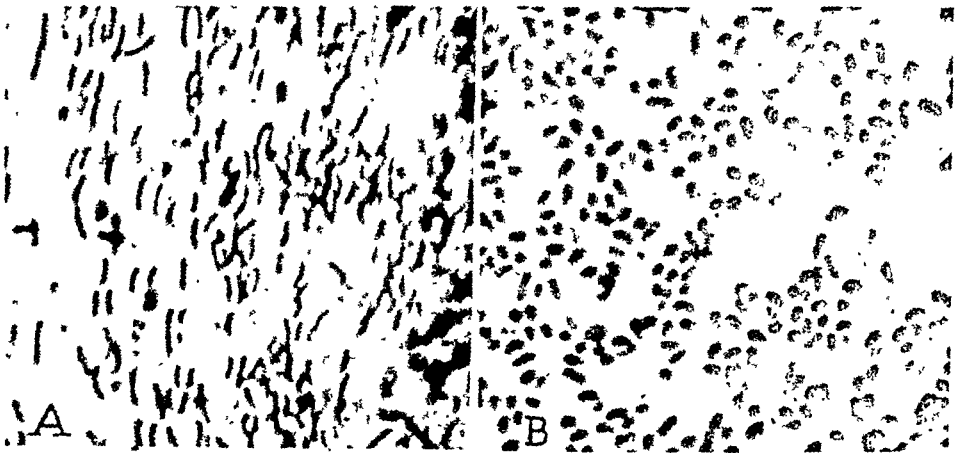


FIG. 5. A. Cholera vibrios as curved rods and other bacteria in the stool before treatment with streptomycin. The bacilli as shown are at times arranged in one direction in the so-called fish-in-stream pattern, probably due to enmeshment in a drawn-out shred of mucus. B. The same strain of *V. comma* in short rods and coccoid forms after one subculture on agar medium.

Two injections of 2000 cc. physiologic salt solution were given within 5 hours, with added solution of sodium bicarbonate, and great improvement followed. The specific gravity of the blood became normal, 1.056, but continual purging required repeated infusions. Vomiting occurred on the second and third days. With rehydration, urine was secreted in increasing volume (chart 1).

Streptomycin was first given orally 27 hours after the onset, dissolved in water, in doses of about 0.3 gm. every 3 hours, a total of about 2.5 gm. in 24 hours. Some was vomited. At the end of this time no vibrios were seen in a rectal smear and other bacteria were rare, yet on culture *V. comma* was still present. Streptomycin was continued in the same dosage for three days, making a total of about 7.5 gm. However, diarrhea persisted for three days more before recovery occurred. The fever shown in the chart, as in other cases, was partly attributed to the injected pyrogenic salt solution. While vibrios were not seen in later rectal smears, *V. comma* was present culturally up to the tenth day. The course was unusually prolonged. Recovery was dated on the sixth day.

Streptomycin obviously had no clinical effect in this patient, but the numbers of vibrios

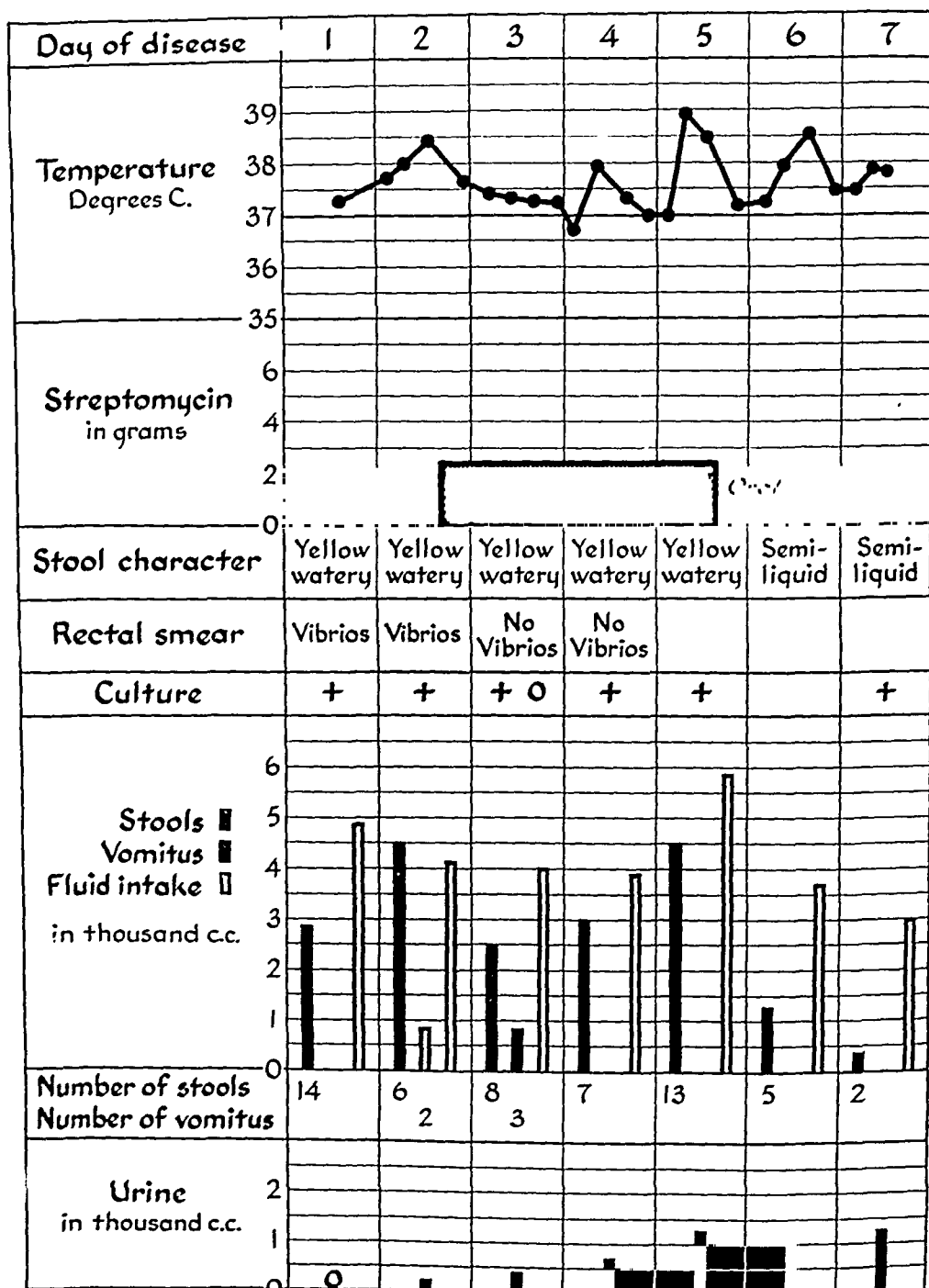


CHART 1 (Case 1). Severe, prolonged cholera. Large amounts of fluid lost in stools and vomitus. Recovery on the sixth day when the stools became fecal and the volume of urine increased to normal after vigorous intravenous and oral rehydration. Vibrios are present in rectal smears before but not after treatment with small amounts of streptomycin, but cultures remained positive until the tenth day.

in the stool were greatly reduced, yet were present on culture as noted in all patients treated with the drug. *V. comma* isolated before treatment and again after 2 days of treatment grew in 1 microgram per cc. of broth but not with 5 micrograms of streptomycin. Since the dosage seemed insufficient, larger amounts were used in the following patients.

*Case 2.* A man aged 31 had a sudden onset of diarrhea and vomiting at 5 A.M. August 10. In the following five hours before admission to the hospital there were ten copious watery stools, and he vomited five times. Severe cramps occurred in his calves. The patient was in a state of collapse, greatly dehydrated, aphonic and lethargic. The specific gravity of the blood was 1.070, the blood pressure 80 systolic, 50 diastolic, the red cells numbered 5.5 million, and the leukocytes 22,000. The stools were like rice water and a rectal smear contained a few comma vibrios proved by culture. Immediate intravenous infusion of 1500 cc. caused prompt improvement, but had to be repeated for another collapse several hours later. Streptomycin was first given about six hours after the onset. To obtain a large amount in the intestine as quickly as possible, an initial dose of one gram was given orally. Thereafter about 0.57 gm. were given every three hours, a total of five grams in the first 24 hours. None was lost by vomiting. During the next 24 hours, five grams more were given in eight divided doses. He received 10 grams in all.

After one day of treatment neither vibrios nor other bacteria were seen in a rectal smear. He appeared and felt nearly well, and the number of stools diminished as shown in chart 2. Recovery was dated on the second day. Cultures from the rectum were positive up to the fifth day, the last from a formed stool. Vibrios before treatment resisted 1 microgram of streptomycin but failed to grow in 5 micrograms.

*Case 3.* A man aged 24, had a sudden onset of voluminous watery stools and soon vomited. In the 15 hours before admission, five stools were passed. Repeated severe muscular cramps came and he was admitted profoundly sick, in a state of collapse with cold, clammy, shrunken skin, cyanosis and a barely perceptible pulse. The oral temperature was 35.8°C. The specific gravity of the blood was 1.070. A smear from the rectal fluid contained myriads of comma bacilli and a culture was positive for *V. comma*. During the first 24 hours, four infusions of physiologic salt solution totalling 7000 cc. had to be given because of repeated collapses and the passage of about 20 stools, measuring 3400 cc., and vomitus of 3900 cc., a total loss of over 7300 cc. of fluid (chart 3).

Streptomycin was given orally after the first infusion. One gram was given as a first dose to attain a large amount quickly in the intestine; thereafter, doses of 0.57 gm. were given at three hour intervals, a total of five grams in 24 hours. Profuse vomiting caused the loss of an undetermined amount of streptomycin. In the second 24 hours, five grams were given, and retained, every three hours in equally divided doses, and on the fourth and fifth days 2 gm. in an attempt to terminate the excretion of vibrios. No vibrios were detected in smears and other bacteria were rare. Cultures for *V. comma* were nevertheless positive until the fifth day. Vibrios isolated before therapy were highly resistant to streptomycin and failed to grow in concentrations of 500 micrograms per cc. of broth.

The patient remained seriously sick during the second day, had 20 rice water stools, vomited ten times and was almost anuric. By the third day, clinical improvement was striking, although eight stools were passed and he vomited four times. The urine volume increased to 1400 cc. and the specific gravity of the blood was normal, 1.054. By the fifth day recovery was established.

*Case 4.* A soldier aged 29, was suddenly seized with profuse purging and vomiting six hours before admission, August 20. The stools resembled rice water, there was no abdominal pain but the muscular spasms in his extremities were severe. He was in a state of collapse, dehydrated, the pulse was barely felt, fever of 39.6°C. developed, and the specific gravity of blood was 1.073. It diminished to 1.054 after the injection of 3000 cc. of fluid. The loss of fluid by stools and vomitus measured 6800 cc. which was balanced by the intravenous injection of over 12,000 cc. of salt solution (chart 4). Smear of the stool before



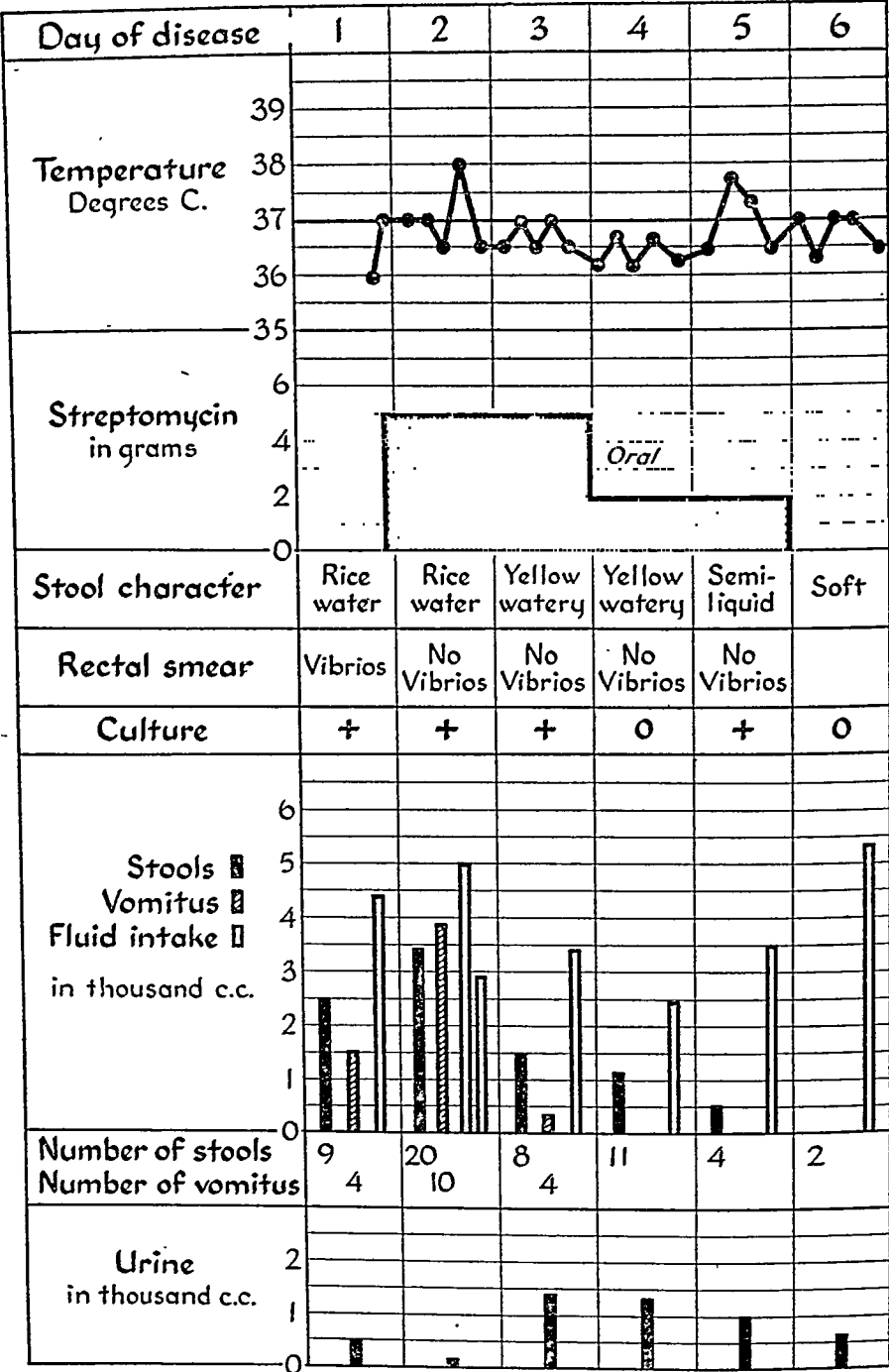


CHART 2 (Case 2). Short attack of cholera. Copious diarrhea and vomiting on the first day only. On the second day urine increased to normal, stools were formed on the fourth day. Fluid after the second day was taken orally. Vibrios disappeared from the stools after the ingestion of 4 gm. streptomycin daily for 2 days, but cultures were positive until the sixth day. The fever probably is caused by reaction to pyrogenic salt solution.

therapy contained myriads of vibrios and a culture was positive. Similar attacks of cholera occurred in quick succession in four other members of his company, all of whom had received anticholera vaccine three months previously.

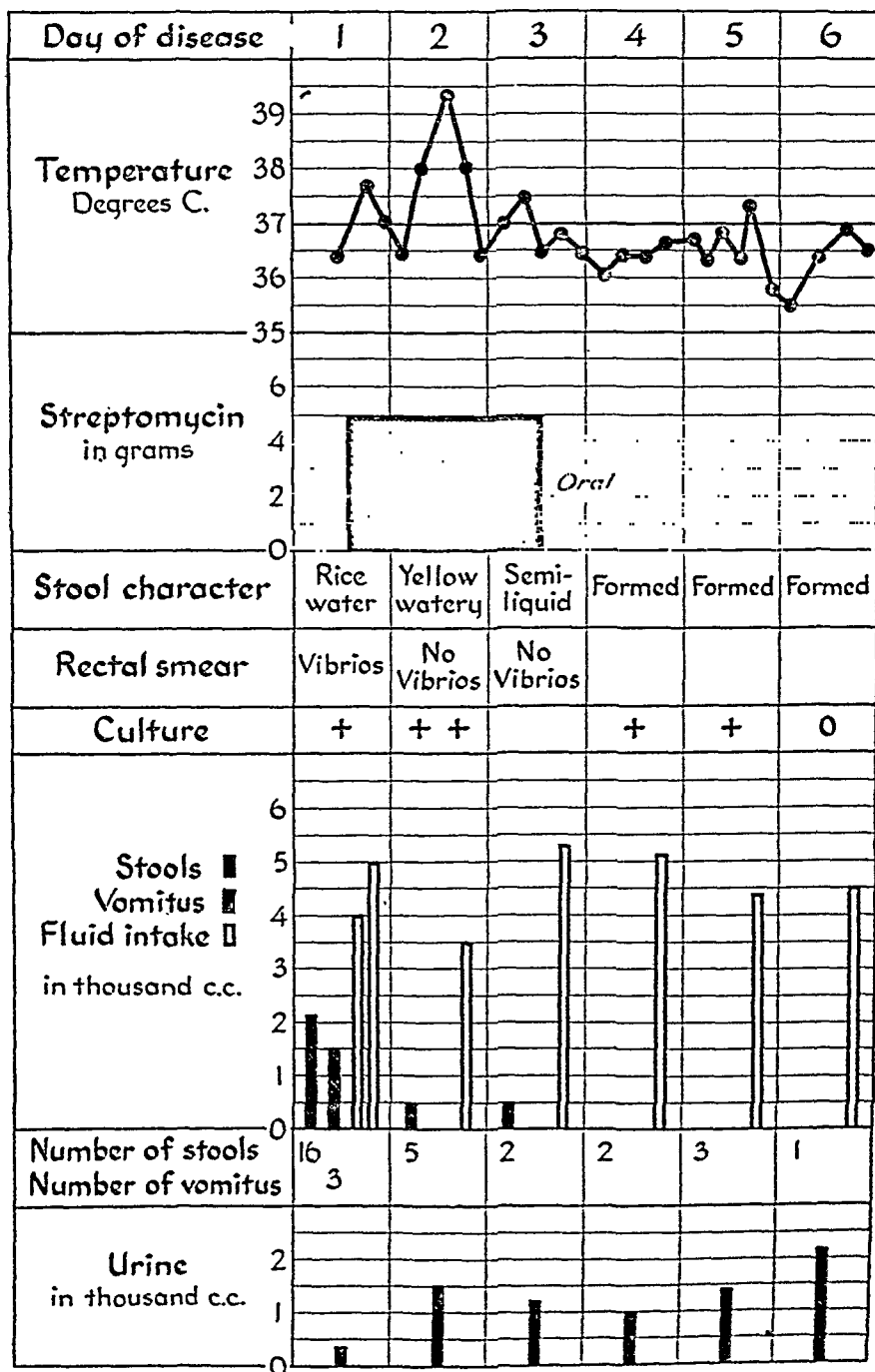


CHART 3 (Case 3). Loss of 4000 cc. of fluid in stools and vomitus the first day, and of 7300 cc. during the second when 8000 cc. of salt solution were injected. Urine volume 150 cc. Vibrios absent in smears after 5 gm. streptomycin daily then 2 gm. daily, but present in stool cultures after therapy.

Streptomycin therapy was started 8 hours after the onset in doses of 3 gm. intravenously and 4 gm. orally for the first 24 hours. The intravenous route was used because of the possibility of the presence of vibrios in the tissues which can only be attacked by parenteral

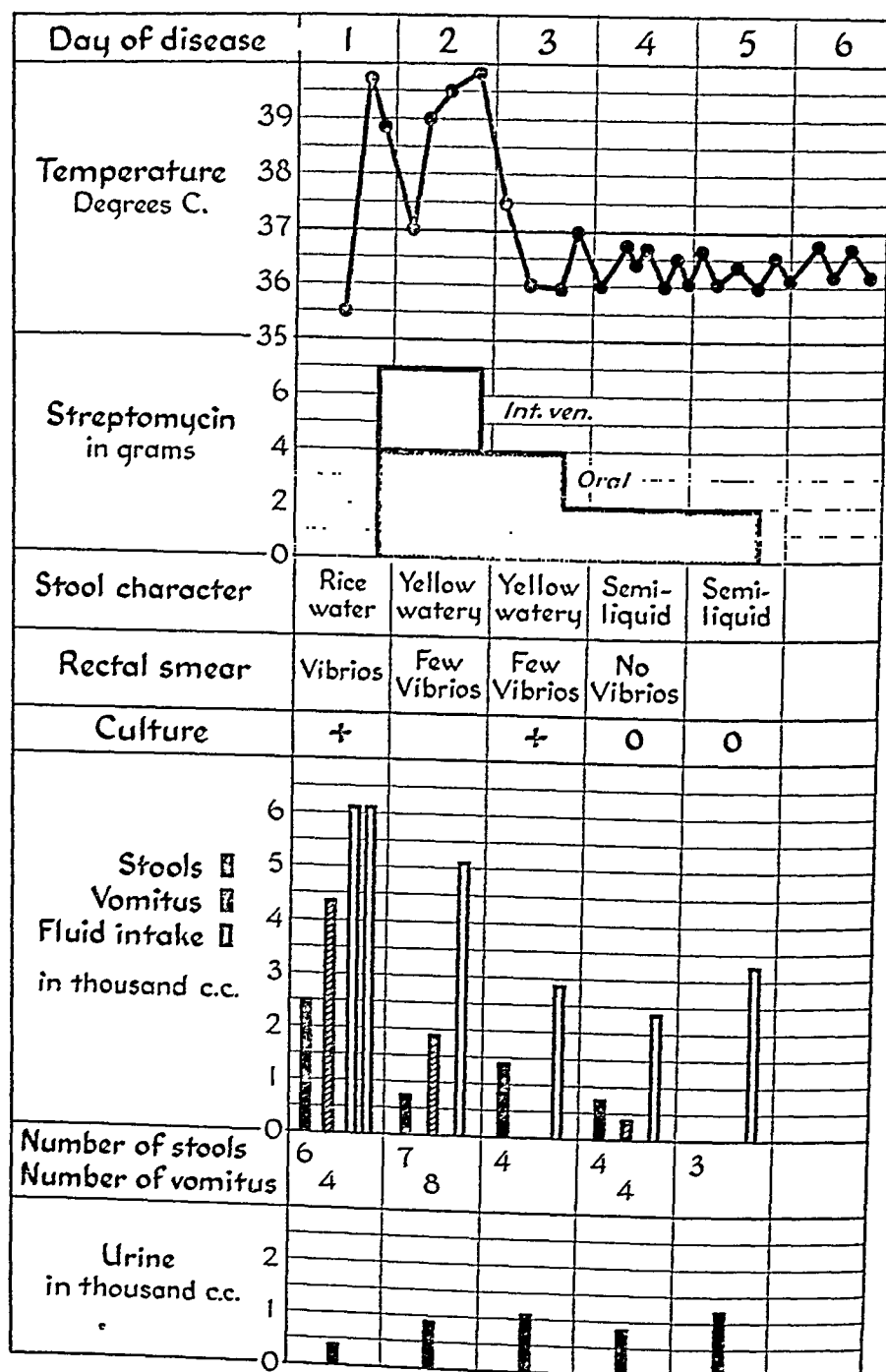


CHART 4 (Case 4). Copious vomiting and diarrhea, and the injection of 12,400 cc. of fluid the first day. Intravenous and oral therapy with streptomycin, 7 gm. in 24 hours, then lesser amounts orally. A few vibrios persisted in smears after therapy. Cultures negative for *V. comma* after the fourth day.

therapy. Because of a mishap 2 gm. ran in in three hours and caused the patient to become comatose, the fever rose to 39.8°C., there was gasping respiration, and an irregular heart rate. Intravenous therapy was stopped and the attack passed in thirty minutes.

Much streptomycin was undoubtedly lost in the vomited fluid and a few vibrios were still present in a smear from the stool after 24 hours of therapy. Recovery occurred on the fifth day. Cultures were negative after the third day. Vibrios isolated before treatment resisted 5 micrograms of streptomycin per cc. of broth but not 10 micrograms.

Case reports of 6 other patients treated with streptomycin need not be given in detail. With a few unimportant exceptions, all were similar. Combined oral and intramuscular therapy was tested in three patients, but the latter in adequate dosage was too painful. Combined oral and intravenous therapy was used in the others with no particular advantage.

#### COMMENT

It is a surprise no doubt to most readers that cholera, if properly treated, is not so fatal as it is said to be. It can be made much less serious than typhoid. In this series of 140 patients, the duration of the attack when vigorously treated was about 4 days and the mortality rate only 5 per cent. In other hospitals at the same time, where treatment was less thorough, the mortality rate was about 16 per cent. It is probable that the mortality rate even among patients not treated at all is less than 50 to 70 per cent as usually stated (8) if all mild, unrecognized cases could be included statistically. Old age, neglect, malnutrition, complications and the concurrence of other diseases reduce the chance of recovery.

Despite the apparent simplicity of the pathogenesis of cholera, which strongly resembles the results of an overdose of a drastic purgative, or of severe food poisoning, its nature is poorly understood. The endotoxin liberated by the disintegration of vibrios in the intestine evidently greatly increases the permeability of the intestinal lining to fluids (9) and serves as an intense hydrogogue to make cholera unique among the infectious diseases. Whether the toxic action is in the central nervous system or, more likely, local in the intestine is unknown, but it is difficult to reconcile local intestinal effects alone with instances of cholera in which vibrios can not be isolated from the stool and in those in which enormous quantities of fluid are vomited, unless there is regurgitation into the stomach from the intestine or unless vibrios are present in the stomach and the toxin affects the mucosa of the stomach as well. It is also difficult to understand why recovery usually comes so promptly after the restoration of salt and water while vibrios persist in large numbers in the stool. Apart from the existence of endotoxin and the apparent toxemia at the onset of disease, the usual absence of fever, the clear mentality and rapid recovery suggest that dehydration, the loss of salts and the acidosis arising therefrom are far more important factors in causing the later signs, symptoms and fatalities.

The average duration of the disease as arbitrarily set in 56 patients treated by rehydration and remineralization alone was 4.6 days. The duration in 30 patients in the same hospital treated by others (10) with sulfaguanidine was 3.8 days, and of 31 treated with sulfadiazine, 3 days. In eight patients selected for the severity of their attack and treated on the first day of disease with streptomycin, the average duration was also 3 days.

It is doubtful if the apparent shortening of the attacks with sulfonamides or

with streptomycin is of significance. In any disease whose earliest stage is as severe as cholera one would expect a period of illness of several days to follow the initial shock, dehydration and demineralization alone even if infection did not persist. Furthermore, if the simple administration of salty water reduces the duration of the disease to a few days and the mortality rate to 5 per cent, there is hardly any need for specific therapy unless it can be shown to abolish the carrier state sooner. Neither the sulfonamides nor streptomycin did. The duration of the presence of viable vibrios during convalescence in the stools of patients not treated with drugs, in patients treated with sulfonamides, and in those treated with streptomycin was about the same. Vibrios could seldom be isolated after

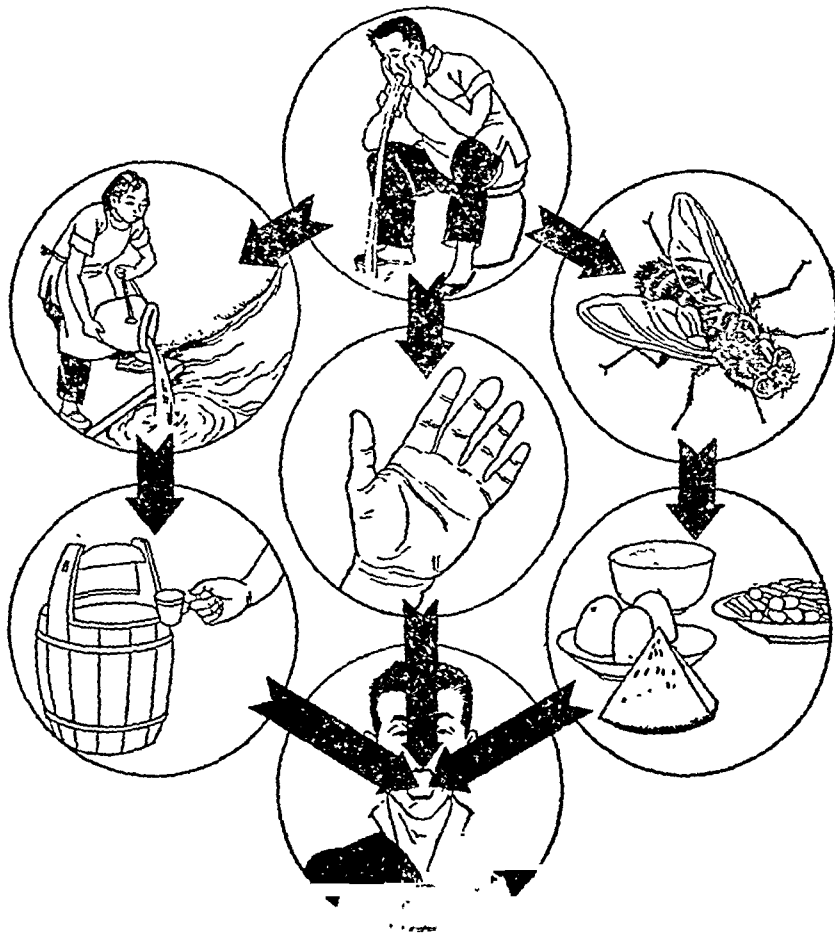


FIG. 6. SIMPLE BUT EFFECTIVE POSTER ADVISING AGAINST DRINKING COLD WATER

the seventh day of disease in any. Vibrios were present in large numbers in smears made from patients treated with sulfadiazine and sulfaguanadine, in contrast with their absence or diminution after therapy with streptomycin. The intravenous injection of plasma as recommended by Naval Officers (11) was not used.

Bacteriologically, more evidence of a specific effect of streptomycin was observed. Vibrios in great numbers were present in stained smears of stools of patients before treatment with streptomycin. In most of these, vibrios were no longer seen after 12 or 24 hours of treatment, yet cultures showed that viable ones were still present. In three patients, vibrios persisted in smears throughout therapy, though greatly reduced in number. As discovered several months later in sensitivity tests made by Dr. W. F. Elias, vibrios from patients in whom they disappeared after treatment grew in broth containing 1 microgram of

# 霍亂傷寒痢疾傳染的途徑



重慶市衛生局印製

## 預防方法

一 不喝生水  
二 不吃露天攤販食品  
三 不吃蒼蠅爬過的食品  
四 不要和病人接觸  
五 要打霍亂傷寒預防針

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FIG. 7. SELF-EXPLANATORY POSTER ILLUSTRATING FORCEFULLY HOW CHOLERA IS SPREAD AND CONTRACTED POSTERS WERE PREPARED AND DISTRIBUTED BY THE NATIONAL HEALTH ADMINISTRATION OF CHINA AND THE CHUNGKING MUNICIPAL HEALTH ADMINISTRATION

streptomycin per cc., but some not in 5 micrograms per cc. and others not at 10 micrograms per cc. Vibrios isolated before treatment in patients in whom they persisted in smears afterward, were highly resistant and grew in concentration of

500 micrograms per cc. of broth. Evidently strains of *V. comma* of the Ogawa variety from different patients of the same epidemic vary greatly in their resistance to streptomycin.

*Prevention.* It is generally believed that the contamination of the public water supply is the chief cause of epidemic cholera, but in the present epidemic, other factors seemed more important in its perpetuation. Water pumped from the local rivers was muddy but contained few bacteria. Contamination more likely took place after the collection of water from the pipe outlets in wooden buckets for distribution or from water dipped from the river's edge. In outlying districts, far from the rivers, water came from wells and ponds, yet from a rapid succession of attacks among certain family groups or in companies of soldiers (case 4, for example) it appeared that infection arose in widely separated places presumably from a carrier or a patient, and was transmitted chiefly by personal contact, similar to the spread of bacillary dysentery (12). Contamination of food and of water by soiled fingers or utensils or by flying or crawling insects carrying infected feces, seemed more likely.

If this be the case, the local problem of prevention is not so much one of expensive, large scale sanitary engineering, though this is desirable, as a change of personal habits generally, to be brought about by education, propaganda and a rise in the standard of living. The present epidemic probably declined spontaneously, but it may have been impeded by the widespread use of educative posters (figs. 6 and 7) throughout the city but especially in the poorer districts, by instruction in preventive measures given in the local newspapers and by radio, and the observance of the advice given. The multiple small procedures of the suitable disposal of excrement in covered privies, the protection of food from insects, the ingestion only of boiled water and of hot food, the washing of hands after defecation, the washing of vegetables and fruit in uncontaminated water, the isolation of patients in screened hospitals or rooms, and the sterilization of their dejecta and soiled linen are of greatest importance. Most of these habits could be achieved even without raising the present low standard of living in the orient. Quarantine of persons exposed to infection, the release of patients only after they no longer excrete *V. comma*, the exclusion of carriers as food handlers and the destruction of insects as outlined elsewhere (13) are also important needs. If these measures were adopted, cholera and other enteric diseases would be exterminated. Anticholera vaccine, though said to be of value, should not be relied upon, lest a false sense of security be aroused.

Perhaps another factor plays a rôle in the susceptibility to cholera. Since the Chinese diet in general is 97 per cent vegetarian (14), the intestinal fluid tends to be alkaline, providing a medium favorable to *V. comma*. The addition of protein in the form of meat, milk, and eggs to the diet may lower the hydrogen ion concentration to a point at which the comma bacillus cannot live (15).

#### CONCLUSION

Cholera, when properly treated, is a disease of short duration and of low mortality. The restoration of salts and water by the intravenous or intraosseous





# STUDIES ON ATABRINE (QUINACRINE) SUPPRESSION OF MALARIA

## I. A CONSIDERATION OF THE INDIVIDUAL FAILURES OF SUPPRESSION

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The intensity of epidemic malaria during the early campaigns in New Guinea and the Solomon Islands in 1942 and 1943 made it imperative that some adequate means of suppressing clinical malaria be found. Knowledge of the efficacy of the only drug available in quantities, atabrine, (quinacrine hydrochloride) was skimpy, inexact, and not directly applicable to the problem of suppression of malaria among nonimmunes. The clearest summary of existing knowledge had been presented by Field (1) but his experiments, which clearly demonstrated the ability of atabrine to suppress the great majority of attacks, dealt with native workers who had been infected over a period of years. Reports by medical officers in the U. S. Army as to the efficacy of atabrine in troops were conflicting and usually negative.

It was at this time that fundamental knowledge concerning the pharmacology of atabrine first became available to the armed forces through a coordinated research program carried out under Shannon's leadership (2). This in turn was based on newer methods of determining the concentration of atabrine in the plasma (3, 4). The direct application of one of these methods (3) to the problems of the control of malaria in the field was the next logical step.

Studies which were carried out along these lines in the Southwest Pacific theater from June 1943 to January 1945 are here reported. Much of the data has been made available to a limited number of individuals through the reports of the Malaria Research Unit. At the start of this work two other programs aimed at obtaining exact data on suppression were under way in the Southwest Pacific. In June 1943 a small but carefully controlled experiment under Colonel M. C. Pincoffs had demonstrated the military effectiveness of 0.6 gram of atabrine per week in suppressing malaria (5). Experiments on individual volunteers in the Australian army under the direction of Brigadier N. Hamilton Fairley (6) showed similar results. Exchange of information with these groups was frequent.

The laboratory evaluation of a suppressive regime is based on the assumption that the antimalarial protection afforded an individual is related to the concentration of atabrine in the plasma. This assumption has been substantiated in general by laboratory studies on therapeutic levels and by field studies on breakthrough attacks occurring during suppression. These individual "failures of suppression," when frequent, lead to the idea that suppression as a whole is not working, even though little may be known about the amount of malaria to be expected were suppression not in use.

<sup>1</sup> On leave from The Rockefeller Institute, Princeton, N. J.

<sup>2</sup> On leave from the International Health Division of the Rockefeller Foundation.

In this first paper we attempt to answer the individual question posed by such failures of suppression and in the following paper to evaluate atabrine suppression in terms of rates and total amounts of infection. Thus in the first we emphasize the clinician's problems, in the second the division or base surgeon's problem. We have concluded that the problem of individual "break-throughs" is one of inadequate dosage—most but not all of which is due to failure to take the required dose. As a background of normal behaviour we studied variations in individuals and in groups known to be taking a standard dose of 0.1 gram per day 6 days a week.

To data of this nature presented by others (2, 6, 7), we add these studies done on troops who had just finished a strenuous campaign and many of whom were heavily infected with malaria. Data on several other programs of therapy are also presented.

#### METHOD

All determinations of atabrine concentrations were done on plasma samples which had been centrifuged twice. The first centrifugation was carried out for 15 minutes within half an hour of the drawing of the blood. Potassium oxalate or sodium citrate was used to prevent clotting. An hour or more sometimes elapsed between the first and second centrifugations, but comparative experiments showed that little increase in plasma atabrine occurred during such an interval. Twice centrifuged samples were kept in the icebox until tested, which in most cases was less than 48 hours.

In the study on the epidemiological significance of atabrine suppression, samples had to be kept for days, often without refrigeration. A few drops of chloroform added to the sample, which was kept in a glass vial with a plastic screw top, prevented bacterial multiplication and did not affect the determination.

All of our early studies (daily individual variation, plasma levels on officer candidates, effect of exercise) were done by the double extraction method. Later studies (break-throughs in New Guinea) had to be done by single extraction because of the lack of lactic acid.

Since there is a wide variation in the concentration attained in different individuals on the same dose, and since there is also variation in one individual between doses, any new regime of therapy must be followed from both these points of view. First we must know how much the concentration varies between doses, and secondly, how much variation there is in a large sample of men.

Opportunity to study the variation in plasma atabrine concentration of soldiers going through a wide range of activity under different suppressive regimes was afforded at the 6th Army Training Center through the courtesy of Lt. Colonel G. G. Duncan, M. C. This camp was established for the purpose of rehabilitating men who had had malaria a number of times. This comprised strictly supervised atabrine administration and an increasingly strenuous program of physical activity under generally ideal conditions. At the end of the program the majority of these men were able to march 25 miles without a break. The com-

bination of strict supervision of drug administration by roster and increasing activity made this group of men ideal for study 13.

### RESULTS

Interest has centered on three types of suppressive therapy carried out by Lt. Colonel Duncan. The first of these was the then standard suppressive therapy for the Southwest Pacific area, namely. 0.1 gram of atabrine 6 times a week. Shannon, et al., (2) have shown that normal active young adults accumulate atabrine in their tissue reservoir very slowly on this regime so that it is a matter of weeks before a tissue plasma balance is reached. Figure 1 shows the gradual accumulation of atabrine in 100 men over the course of ten weeks. The first

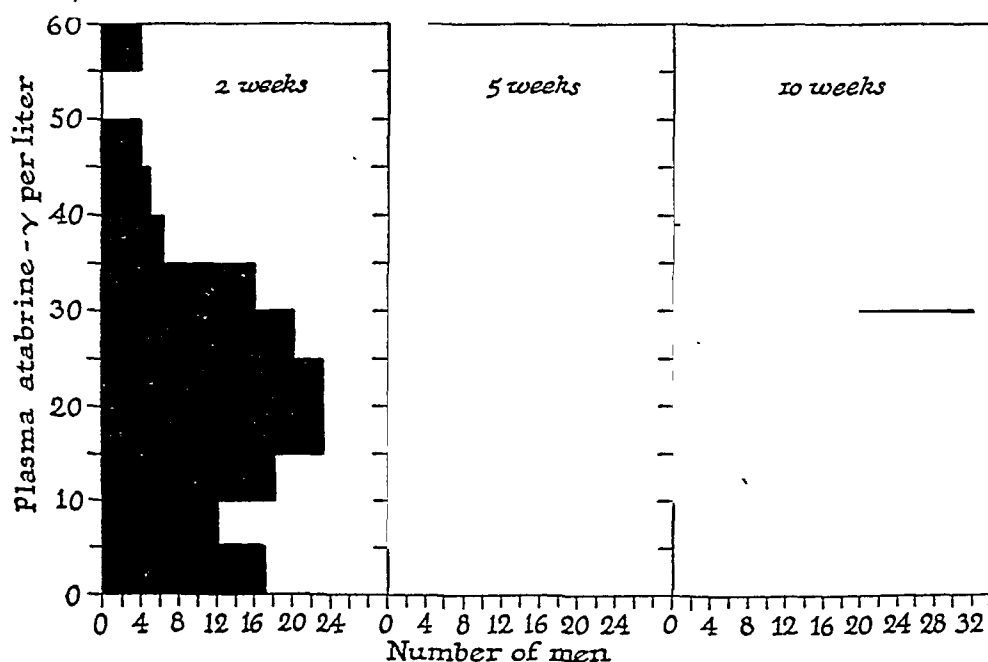


FIG. 1. INCREASE OF PLASMA ATABRINE CONCENTRATION IN 100 MEN DURING RIGIDLY SUPERVISED ADMINISTRATION OF 0.6 GM. PER WEEK

group of men had been on supervised atabrine for about two weeks. Unfortunately, some of these men had stopped taking atabrine for variable periods of time, and some of them were still taking suppressive atabrine when admitted to camp. Thus many of the levels in this group are much higher than they would have been had all of them been receiving atabrine for only two weeks. The second group is composed of many of the same men taken five weeks after they had started suppressive atabrine. The third group is composed of another hundred men who had been taking suppressive atabrine for ten weeks and who had just finished the third phase of their training. These levels were taken the day after their 25-mile march. We may conclude that the men accumulated atabrine in their tissues and plasma despite a simultaneous program of rigid training. Two of the men in the second group had a positive smear (1 parasite per 500

w.b.c.) and no symptoms. In the survey of the third group of men who had been on atabrine for ten weeks, one man (not included on the chart) was found who had a positive smear (850 rings per 500 w.b.c.) and an atabrine plasma level of 7 gamma per liter. Upon questioning, he admitted that he had missed taking atabrine for five days, and that he did have symptoms of malaria. All of these results apparently indicate that there is some accumulation of atabrine in the body after five weeks. Experiments by others, however, indicate that this does not continue much beyond six weeks (2, 7).

#### INDIVIDUAL VARIATION

In order to follow the daily variation in levels while taking one tablet per day, five men were bled every 4 hours between doses, one group after three weeks of

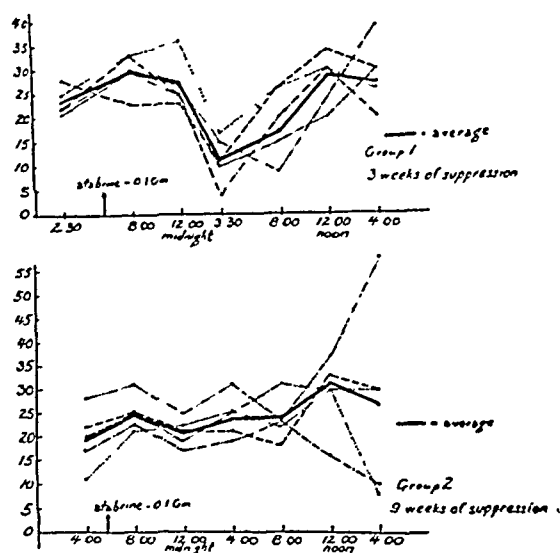


FIG. 2. VARIATION IN ATABRINE LEVELS DURING THE DAY IN TWO GROUPS OF FIVE MEN TAKING 0.1 GM. 6 TIMES PER WEEK

suppressive therapy, the other at the end of the program (10 weeks). It will be noted (see fig. 2) that in both groups the individuals attained a concentration of 30 gamma at some time during the day. These studies would indicate that most of the men included in figure 1 who had inadequate levels at that time probably had adequate amounts of atabrine in their plasma at other times of the day. This in turn indicates that the results of an individual reading must be interpreted with considerable caution.

Two other suppressive regimes were studied. When men were given 0.4 gram or 0.5 gram every three days they had levels which were the same, or even greater, just before their next dose as men receiving the standard regime. Figures 3 and 4 show that the levels were more than adequate most of the time between the doses.

There was considerable daily variation in levels on all regimes, a variation not related to the time of the dose. This was at first attributed to daily activity,

since men taking 0.1 gram of atabrine per day were found to have lower levels after short periods of activity. As atabrine accumulated in the tissues, this reduction in level became less apparent.

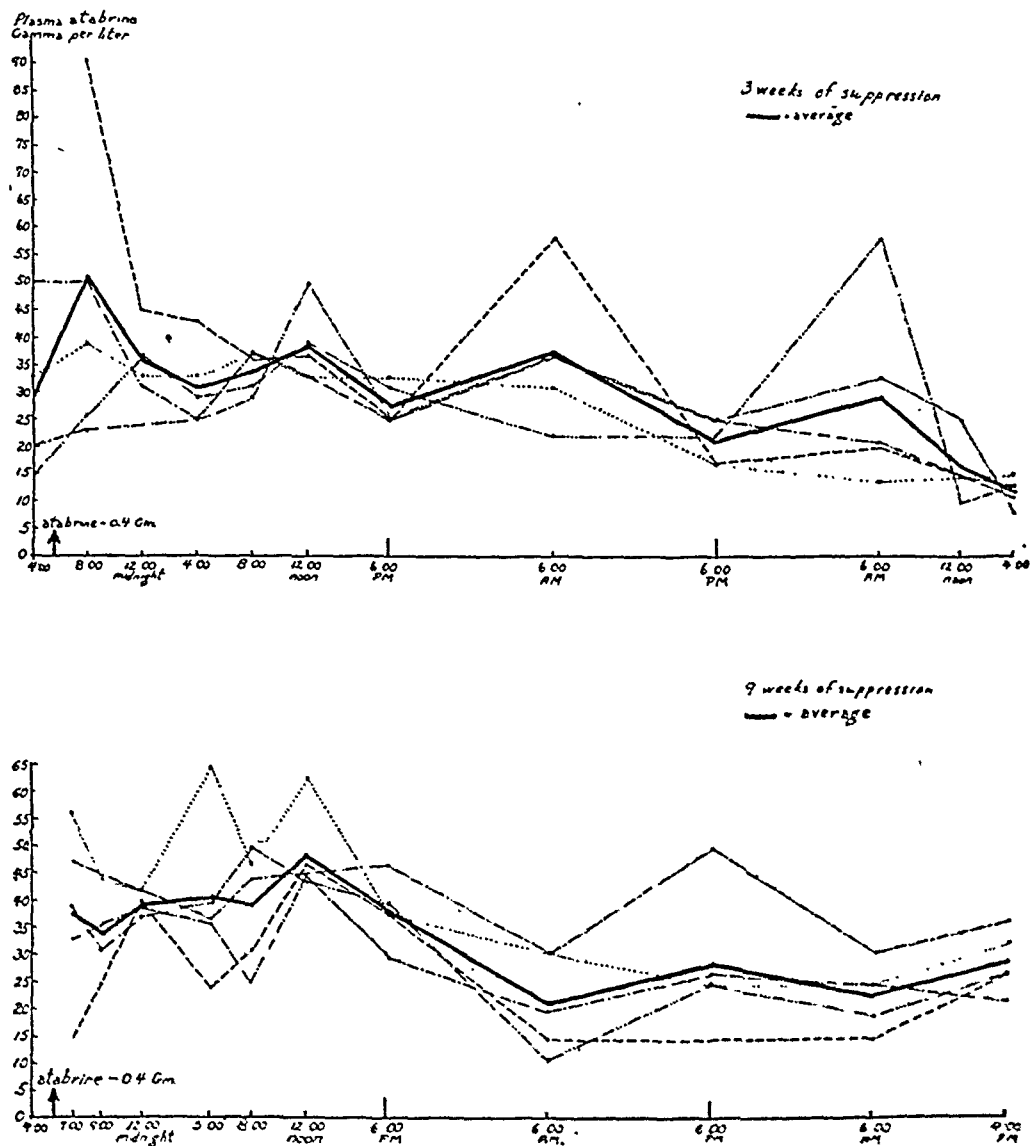


FIG. 3. VARIATION IN ATABRINE LEVELS IN TWO GROUPS OF FIVE MEN TAKING 0.4 GM. EVERY 3 DAYS

In four separate experiments using 10 to 15 men in the experimental and control groups, we twice found an appreciable difference between the average (table 1). The exercise group either made short marches or spent the morning engaged in athletics. The controls were forced to remain near the tent, and most of the time they stayed on their bunks. Studies over a longer period of time (two weeks) with frequent atabrine determinations, showed that there was no differ-

ence between men who carried out the normal camp routine and those forced to remain at rest most of the day. Finally, the continued accumulation of atabrine in the plasma despite increasing activity indicated that activity had no great influence on the concentration of atabrine in the plasma.

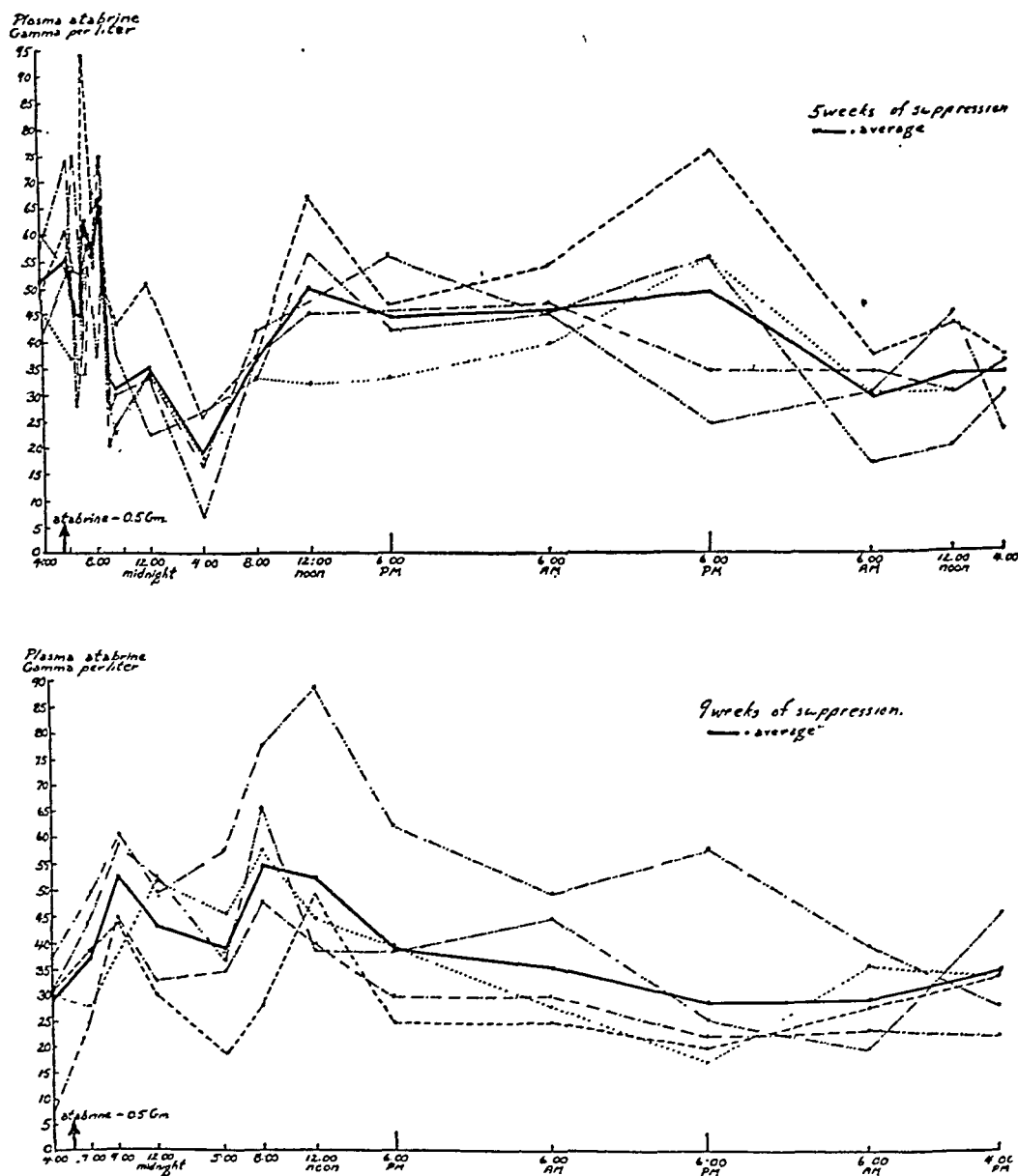


FIG. 4. VARIATION IN ATABRINE LEVELS IN TWO GROUPS OF FIVE MEN TAKING 0.5 GM. EVERY 3 DAYS

A comparison of the variation in levels between doses for the different regimes at the beginning of the training period and at the end showed that with time there was less variation. The evidence at present is, however, too slight to relate it to the accumulation of atabrine in the tissues.

## BREAK-THROUGHS

With this background of normal behaviour of atabrine plasma levels we may take up the more crucial problem of failures of suppression or "break-through" attacks. We have arbitrarily divided them into three classes, relapses, primary attacks of vivax, and primary attacks of falciparum malaria. It is well to define the term "break-through." It is a clinical attack of malaria (the diagnosis confirmed by a positive thick smear) developing in an individual presumably taking suppressive medication. Furthermore, the attack must respond to therapy. We thus by definition exclude transient slight parasitemia in the

TABLE 1

*Effect of exercise on plasma atabrine concentration*

	NO. MEN STUDIED	Experiment	AVERAGE PLASMA ATABRINE CONCENTRATION		
			Before	Immedi- ately after	Several hours after
			<i>gamma per liter</i>	<i>gamma per liter</i>	<i>gamma per liter</i>
Exercise.....	15	5-mile hike	18.5	11.5	13.3
Control.....	10	rest	21.5	22.0	18.0
Exercise.....	15	Athletics	28.4	29.6	43.8
Control.....	15	rest	32.7	24.6	38.7
Exercise.....	10	25-mile hike	27.6	34.7	35.2
Control.....	10	rest	29.4	44.3	38.6
Exercise.....	15	3-hour hike	40.7	33.3	30.0
Control.....	14	rest	37.7	30.1	32.0

absence of symptoms, and also exclude the not infrequent cases of dengue or scrub typhus which also occasionally showed a very few parasites when careful search was made. The intensity of parasitemia in these cases was not great enough to be picked up on routine hospital examinations and therefore such cases did not often cause confusion.

## RELAPSES

Under this heading we will not include the problem of recrudescing fever during the course of treatment of an acute attack, but will deal solely with cases of recurrent vivax attacks developing weeks after the original attack despite supposed continued suppressive therapy. Clinical malaria is rare in any large group of individuals who are known to have received 0.6 gram of atabrine per week with regularity. The great majority of cases admit to irregular and inadequate regimes.

The problem is well illustrated by a study of 75 officer candidates, all of whom had been exposed to malaria in New Guinea during the early campaigns and at the time of this study were training in a nonmalarious area of Australia. Many

had been taking atabrine for six months to a year, despite which some had had as many as six recent attacks of malaria. The men in this group almost uniformly stated that they were taking 0.1 gram of atabrine every day. As the first plasma levels were lower than expected, the men were questioned more closely. With the demonstration to many individuals that inadequate dosage resulted in lower levels, more accurate histories were obtained. Finally, one-half (13 of 28) of a small group which was closely questioned, admitted that they had forgotten to take atabrine for several days to a week during the three weeks

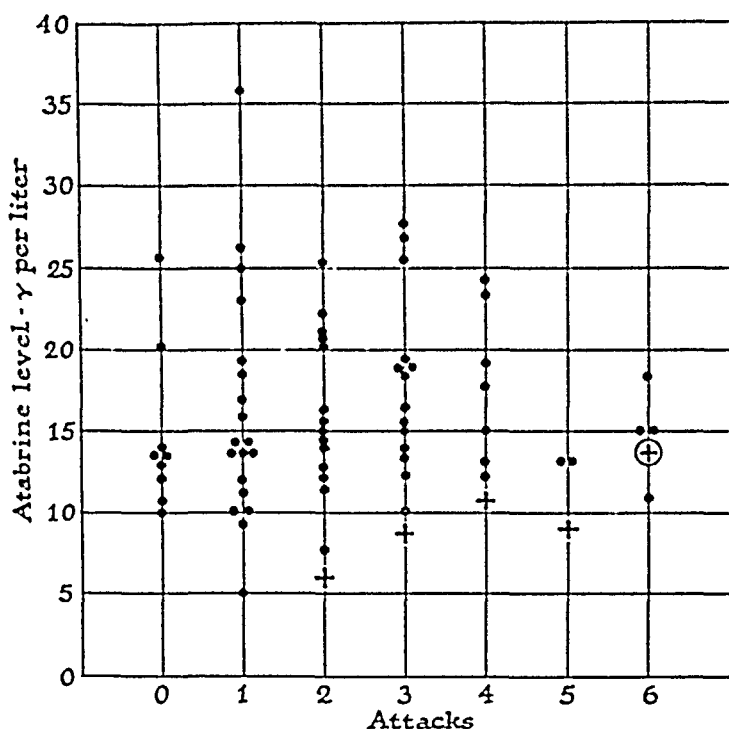


FIG. 5. PLASMA ATABRINE LEVELS IN A GROUP OF OFFICER CANDIDATES NOT ON SUPERVISED ATABRINE

+ represents men who had clinical malaria. ⊕ represents a man who had positive smear but no clinical attack.

study. Figure 5 shows the average level for the different individuals and the relation of those with malaria to the rest of the group.

In figure 6 we present comparisons between the levels of individuals known to be taking 0.1 gram of atabrine 6 times per week under rigid supervision and those of individuals with relapses who said that they were taking that much or more. Interpretation of the data is undertaken in the next section.

#### PRIMARY VIVAX AND FALCIPARUM ATTACKS

In this section we include a consideration of attacks of malaria developing in endemic areas, most of which were the first attack for the individual. The plasma atabrine levels found in these break-throughs eliminate the possibility



that resistant strains account for any large proportion of cases (fig. 6). Secondly, they show that for those who have actually been taking their atabrine the problem is one of individual dosage. All of the men presented in these charts claimed under careful questioning that they had been taking their atabrine regularly.

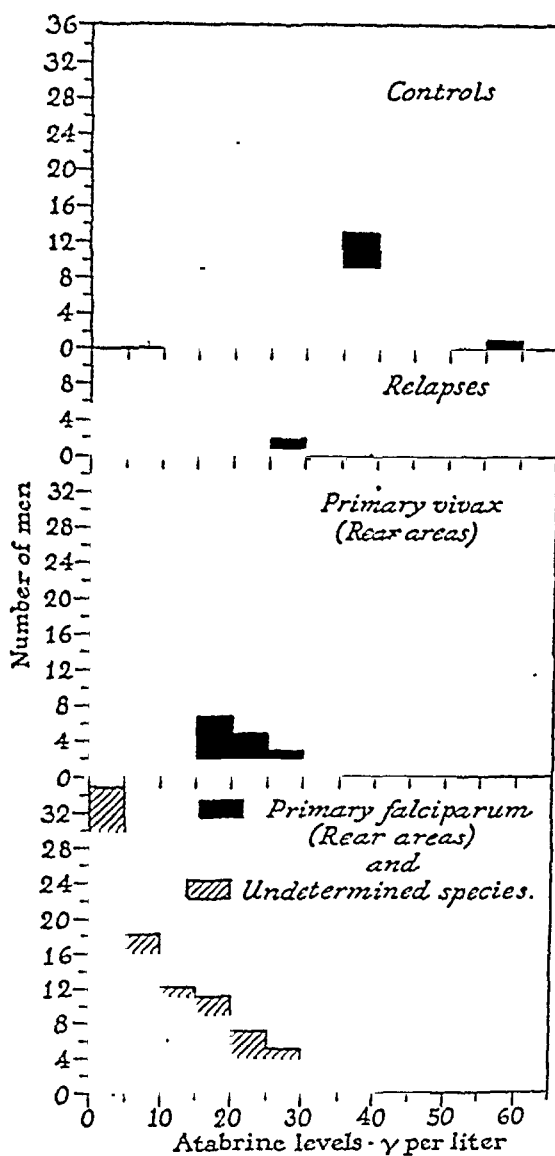


FIG. 6. PLASMA ATABRINE LEVELS IN BREAK-THROUGHS COMPARED WITH ADEQUATELY SUPPRESSED MEN

Many were eliminated from this series because of gaps in suppressive treatment during which quinine was taken, or long furloughs during which atabrine was not taken and suppression had been resumed only a week or two preceding the clinical attack. Because of the slowness with which the atabrine level attains the maximum on a constant dosage, the average man cannot be considered to be effective.

tively protected until he has taken at least 0.6 gram per week for two to four weeks. Another large group was eliminated because of irregular use of atabrine or regular use of quinine.

It took over five months to accumulate 152 valid cases of falciparum and vivax malaria break-throughs in both Australian and American troops from different parts of New Guinea where transmission took place. The paucity of such cases illustrates the fact that in base areas suppression of malaria is an administrative problem.

The concentration of atabrine in the plasma of these break-throughs when measured shortly after admission to a hospital varied from 0 to 30 gamma per liter. The figures have been grouped in 5-gamma classes because the error of

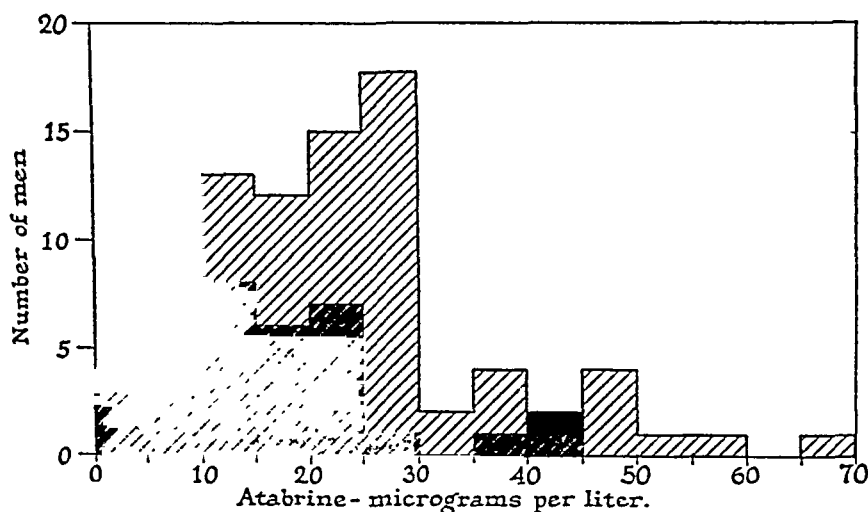


FIG. 7. A COMPARISON OF ATABRINE LEVELS IN BREAK-THROUGHS WITH ATABRINE LEVELS OF MEN IN THE BASE NOT SUFFERING FROM MALARIA

Black—Break-throughs of clinical malaria occurring during atabrine suppression. Mean level 11  $\gamma$ /liter.

Shaded—A group of men on atabrine suppression and showing no signs of clinical malaria. Mean level 21  $\gamma$ /liter.

the method prevents us from attaching any significance to variations as much as 5 gamma. At first glance it would appear that there was no significant difference between the falciparum and vivax cases. However, there is now increasing evidence from controlled laboratory studies (6, 8) that a larger dose of atabrine is necessary for successful treatment and suppression of falciparum malaria. This claim has also been made for field studies in the South Pacific theater by Baker (9).

Examination of the distribution of our "break-throughs" shows that there are twice as many cases of *P. falciparum* with levels between 6 and 20 gamma as there are cases of *P. vivax*. These might well be considered true failures of suppression. Our data may then be considered to agree with the laboratory studies. However, variation in atabrine levels from time to time from individual to individual and the problems of drug administration are so great that any difference between strains is of no practical significance in the field.

One infection of *P. malariae*, which is not presented in the chart, had a level of 9 gamma. The cases in which the species of malaria was not determined have been included under *P. falciparum* infections but represent only a few infections.

In this chart are also included the levels obtained on 100 men who were effectively suppressed although heavily infected (80 per cent relapsed 6 to 8 weeks after suppression was stopped). None of these fell below the 10-gamma mark. Yet the normal distribution curve of the suppressed individuals (fig. 6) clearly indicates that with a much larger series there would be a few individuals below ten. Thus the fact that a man has a low level cannot be taken as proof that he has not taken his atabrine regularly.

Two-thirds (96 of 152) of the "break-throughs" had concentrations of 10 gamma or less; 23, or 15 per cent, had levels above 20 gamma. Despite the considerable overlap between the "break-throughs" and the protected individuals, we believe that the concentrations obtained in the latter group usually are protective. A number of clear-cut experiments by others as well as by ourselves have shown that 0.6 gram of atabrine per week protects the vast majority of individuals (5, 6, 10).

An additional 44 authentic break-throughs were accumulated by one of us during three months at a single base in New Guinea (Finschhafen). The data on the atabrine levels of these men is presented in figure 7, together with data on atabrine levels of an unselected group of men at the same base who were also taking 0.6 gram or 0.7 gram of atabrine weekly under good supervision.

#### DISCUSSION

In order to explain the apparent contradictions in these results, it is first necessary to consider some of the factors involved in the use of suppressive atabrine. The original investigations on plasma atabrine levels were founded on the supposition that it would be possible to determine accurately the suppressive level in a particular area. It was expected that the problem of suppression would resolve itself into one of keeping the level of atabrine above the point at which no break-throughs occurred,—30 gamma per liter. The idea that an effective level could be determined by a study of the break-throughs alone ignored a number of complicating factors.

Field, who was the first to demonstrate the value of suppressive atabrine, says: "The effectiveness of drugs used in a malarious population for prophylaxis probably varies with the degree of existing immunity" (1). We have found in our work that the slight degree of immunity conferred by a series of previous attacks will, during suppression, prevent infected individuals from maintaining enough parasites in their blood to be picked up on thick smear examination.

There is a graduated response to increasing concentrations of antimalarial drugs. This has been demonstrated for the effect of quinine on *P. lophurae* in ducks (11), and it applies as well to atabrine and its effect on human malaria (8). It is also worth considering the life cycle of the parasite itself in man. In periods between relapse there is apparently some stage of the parasite which is resistant to atabrine; yet later on, when it has developed to the stage which multiplies in the peripheral blood, it is susceptible to atabrine and may be most susceptible

during the dividing stage. Here the drug effect is graded within the host itself. We cannot with our present knowledge know how far back in the cycle it is desirable and possible to push the atabrine effect. Finally, with this drug, as with others, the level of effectiveness cannot be considered separately from the time of exposure (8). A short exposure to high concentrations of atabrine has an almost immediate effect on the parasite, but the effect is partly reversible (12).

Our measurements of atabrine concentrations in break-throughs are made after the subject has developed symptoms; in other words, after the incubation period during which the parasites multiplied at sub-clinical levels. We have no guarantee that the atabrine concentration observed in the plasma on admission to the hospital is the same as that during which the parasitemia developed. Indeed, it has been demonstrated that the atabrine level may suddenly change when there has been no change in dosage (fig. 2 and 4).

An individual's plasma atabrine level may vary during the day and thus a survey of a group of men at one time does not represent the total experience of that group of men for that day. Even when the data are over simplified by statistical considerations there is still a calculated *average* rise of 10 gamma per liter 8 hours after taking a dose of 0.1 gram (7). Since this rise is sustained for some time, it cannot be considered as without effect.

To summarize: A careful consideration of the distribution of suppressive and break-through levels does not indicate the need for a dosage greater than 0.7 gram per week. In making this statement, we have ignored those few break-throughs which showed concentrations above 30 gamma because of the known tendency of some men to take a few extra tablets when they feel poorly. Omitting these cases, there is still an overlap of about 20 gamma per liter between the susceptible and protected groups. Five gamma of the overlap can be explained by error in the method of determining concentrations. At least 10 gamma is explained by the average daily variation, not to mention individual variation.

#### SUMMARY

Studies on the efficacy of atabrine suppression of malaria in American and Australian troops in New Guinea and Australia from June 1943 to January 1945 are presented. The concentration of atabrine present in the plasma of those developing malaria despite supposed suppression is compared with data from adequately suppressed troops. This comparison shows that clinical malaria develops in the presence of inadequate amounts of atabrine. The concentration of atabrine present in "break-throughs" is the same in chronic relapses and "primary" attacks of malaria. It is possible that *P. falciparum* requires in the field a slightly greater amount of atabrine to be completely suppressed. Our data would support but not establish such a contention. The difference is not great enough to be of practical importance.

The difficulty of obtaining any large number of cases of malaria who consistently claim to have regularly taken 0.1 gram or more of atabrine every day and who have nevertheless developed clear-cut malaria emphasizes the fact that atabrine suppression is mainly a problem of drug administration. However,

the development of malaria in an individual can not be taken as definite proof that that individual has failed to take atabrine regularly even if his drug level is low. Variation of drug level obtained with a standard dose is so great that, granting concentration is related to protection, a small percentage would be unprotected by the standard dosage of 0.7 gram of atabrine per week.

The evaluation of several different dosages of atabrine by studies of the concentration of atabrine in the plasma under different conditions and at different times fails to indicate any need for a dosage greater than 0.1 gram per day. Atabrine levels are adequate between doses when 0.4 gram or 0.5 gram is given twice a week. This dosage scheme must be judged on the relative value of giving the drug fewer times during the week, the increased nausea produced by larger doses, and the greater hazard of more serious reactions when such amounts of atabrine are ingested over a long period.

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# LONGEVITY OF KILLING EFFECT OF DDT FOR MOSQUITOES CONTACTING SCREEN WIRE PAINTED WITH DDT SOLUTIONS\*

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Varying concentrations of DDT in solution in different solvents were tested by painting copper screen wire of 18 meshes per inch. Tests were made at varying time intervals after painting, the tested wire being kept under differing conditions of exposure.

The aims of the experiments were to demonstrate the following points;

1. The length of contact with the painted wire required to kill within selected subsequent arbitrary time period.
2. The original lethal effect of the newly painted wire and the period over which subsequent lethal effect would persist.
3. The effect of different DDT solvents on primary lethal effect, and the duration of lethal effect as modified by different solvents.
4. The effect of varying concentrations of DDT in different solvents on the primary lethal effect, and the duration of lethal action.
5. The influence of different types of "weathering" on the lethal effect of DDT in solution.

The practical aim of the study was directed toward securing evidence regarding the best manner of treating screen wire with DDT for use on Army buildings to effect, by residual DDT action, killing of anopheline mosquitoes making contact with the painted wire, and to determine the solvent which produced the greatest longevity of such action.

From the experiences gained by previous workers, from a purely arbitrary basis, and governed by supplies available, several possible solvents were chosen; namely, acetone, kerosene, Diesel oil #2, heavy American mineral oil and SAE oil #30 and #50. (For further information of solvents and materials see Appendix No. 1.)

Solutions were made so that a given amount of the solution would represent various concentrations of DDT per square foot when painted on the wire. Painting the wire was effected by daubing the measured quantities with cotton swab on the standard size piece of wire to be used in the test. All the solution containing the known amount of DDT was utilized and distributed on the wire as evenly as possible.

\* Report of Experiments at the Army School of Malariology Designed to Demonstrate Effects on Mosquitoes Making Contact with Various Solutions of DDT on Screen Wire under Various Conditions.

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## PROCEDURE OF EXPERIMENT

During the preliminary work it became obvious that long periods of contact with DDT did not appear always to be necessary to produce a killing effect on mosquitoes. Instantaneous contact seemed to suffice for this end. Accordingly, a "contact technique" was developed whereby mosquitoes could be exposed to the DDT painted wire for momentary contact. This light exposure to DDT has made it possible to evaluate the relationship of dosage and potency of DDT

TABLE 1

*Summary of mortality rates produced by exposure of Anopheles albimanus adults to screens painted with varying strengths of DDT and weathered outside for varying periods of time, with "control" group, to which no DDT had been added to the solvent, recorded*

A Series—Solvent: Diesel Oil #2

DDT RESIDUE MG/ft. <sup>2</sup>	PERIOD OF CONTACT	PERIOD OF DAYS SCREENS WEATHERED WITH MORTALITY RATE OF MOSQUITOES EXPOSED TO THEM															
		1 day	8 days	15 days	22 days	29 days	36 days	43 days	50 days	57 days	64 days						
		Mortality (in percentage) in time after exposure to weathered screens															
		in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.
200 mg.	4 cont. 30 sec. 60 sec.	100	100	40	40	10	25	0	5	0	5	Revitalized 25	45	0	20		
100 mg.	4 cont. 30 sec. 60 sec.	80	95	30	30	5	15					Revitalized 15	20	0	5		
				65	80	30	40	0	10		5					5	25
																20	30
																25	35
50 mg.	4 cont. 30 sec. 60 sec.	80	90	10	35	0	0	0	0	0	10						
Control	4 cont.	0	25	0	5	0	10	0	0	0	5	Revitalized 0	5	0	0		

in its effect on mosquitoes under varying conditions. (For procedure of contact procedure see Appendix No. 2.)

Early experience demonstrated that solutions of DDT in volatile solvents, which, on evaporation left DDT in a dry crystalline state, did not produce lethal effect on mosquitoes which had received only four instantaneous contacts with the painted surface. Accordingly, acetone and kerosene, which evaporated relatively quickly, were discarded as solvents in this study.

Suitable time intervals (subsequent to exposure) had to be chosen over which



mortality would be recorded. Mortality among the mosquitoes was routinely recorded 2, 5, 24, and 48 hours after exposure. The findings of the 5 and 24 hour periods are presented here. Mortality within 5 hours represents a rigid test.

Each test for each screen, painted with a particular solvent and a certain concentration of DDT "weathered" over a definite interval, was tested with 20 mosquitoes. For each test run with a particular solvent and DDT, a control

TABLE 2

*Summary of mortality rates produced by exposure of Anopheles albimanus adults to screens painted with varying strengths of DDT and weathered outside for varying periods of time, with "control" group, to which no DDT had been added to the solvent, recorded.*

B Series—Solvent: SAE #30

DDT RESIDUE MG/ft. <sup>2</sup>	PERIOD OF CONTACT	PERIOD OF DAYS SCREENS WEATHERED WITH MORTALITY RATE OF MOSQUITOES EXPOSED TO THEM																			
		1 day		8 days		15 days		22 days		29 days		36 days		43 days		50 days		57 days		64 days	
		Mortality (in percentage) in time after exposure to weathered screens																			
		in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.
200 mg.	4 cont. 30 sec. 60 sec.	90	100	85	100	60	95	55	90	25	45	20	30	20	20	15	20	0	10		
															25	40	25	55	15	35	
100 mg.	4 cont. 30 sec. 60 sec.	60	75	65	85	30	75	35	55	5	10	10	20	5	15						
								50	80	60	85	30	60	0	15	5	10	0	0	0	10
50 mg.	4 cont. 30 sec. 60 sec.	10	55	20	45	25	55	10	25	5	15	5	5	5	5						
																	0	0			
Control	4 cont.	0	20	0	0	0	15	0	10	0	0	0	0	0	0	0	0				

alone, with no DDT added. From the two sets of screens, test and comparison, mortality at subsequent time intervals could be then compared, DDT being the only recognized variable

series of 20 mosquitoes was tested, as comparison, with every point of the procedure identical with the test run except that the screen was painted with the solvent

*Note:* It is unavoidable that some few tests present discrepancies—

1. When the measuring rod is a living entity and mortality constitutes the final measure of results;

2. When physical difficulties prevent the uniform application of the killing agent to all portions of the test surfaces;

3. When meteorological conditions differ on different test days and over different test periods;

TABLE 3

Summary of mortality rates produced by exposure of *Anopheles albimanus* adults to screens painted with varying strengths of DDT and weathered outside for varying periods of time, with "control" group, to which no DDT had been added to the solvent, recorded.

C Series—Solvent: SAE #50

DDT RESIDUE MG/ft.	PERIOD OF CONTACT	PERIOD OF DAYS SCREENS WEATHERED WITH MORTALITY RATE OF MOSQUITOES EXPOSED TO THEM																			
		1 day	8 days	15 days	22 days	29 days	36 days	43 days	50 days	57 days	64 days										
		Mortality (in percentage) in time after exposure to weathered screens																			
		in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.
200 mg.	4 cont. 30 sec. 60 sec.	55	85	50	70	40	100	45	70	30	65	30	55	25	40	15	25	10	15	0	10
														55	90	40	65	30	60	25	45
100 mg.	4 cont. 30 sec. 60 sec.	30	55	20	30	30	80	5	15	10	20	0	5								
								20	50	30	60	30	45	30	60	5	25	10	25	10	20
50 mg.	4 cont. 30 sec. 60 sec.	35	40	15	30	15	65	5	25	0	10	0	10								
														0	10						
Control	4 cont.	0	5	0	15	0	0	0	10	0	0	0	0	0	5	0	5	0	0		

TABLE 4

Summary of mortality rates produced by exposure of *Anopheles albimanus* adults to screens painted with varying strengths of DDT and weathered outside for varying periods of time, with "control" group, to which no DDT had been added to the solvent, recorded.

D Series—Solvent: Mineral Oil

DDT RESIDUE MG/ft.	PERIOD OF CONTACT	PERIOD OF DAYS SCREENS WEATHERED WITH MORTALITY RATE OF MOSQUITOES EXPOSED TO THEM																	
		1 day	8 days	15 days	22 days	29 days	36 days	43 days	50 days	57 days	64 days								
		Mortality (in percentage) in time after exposure to weathered screens																	
		in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.	in 5 hrs.	in 24 hrs.
20 mg.	4 cont. 30 sec. 60 sec.	85	95	70	100	55	100	40	75	35	65	25	55	0 25	10 40	0 10	0 15		
100 mg.	4 cont. 30 sec. 60 sec.	65	90	35	80	40	90	30 60	45 65	20 40	30 50	15 30	35 50		10 25		0 10	10 25	
50 mg.	4 cont. 30 sec. 60 sec.	65	90	40	75	40	100	30	55	30	35	5	5		10 20		0 5		
Control	4 cont.	5	20	0	20	0	5	0	0	0	0	0	0	0	5	0	5	0	0

4. When "weathered" surfaces, though receiving the same general natural exposure, may be affected by individual factors causing loss of potency, such as a gust of wind, or rain, or a blow by an object blown by a wind.

However, such discrepancies in these experiments have been surprisingly few.

The tabulated results of these experiments are recorded in the appended

TABLE 5

*Mortality produced by DDT in diesel oil #2 applied to screen wire at rate of 100 mgs. per square foot over successive periods of weathering inside and outside of buildings*

SCREEN NO.	AGE SCREEN	SOLVENT	DDT RESIDUE MGS. PER SQUARE FOOT	PERIOD OF CONTACT	% MORTALITY AT VARYING PERIODS AFTER EXPOSURE			
					2 hrs.	5 hrs.	24 hrs.	48 hrs.
Screens weathered outside								
A-11	1 Day	Diesel Oil	100	4 Cont.	55	80	95	95
A-12	8 Days	Diesel Oil	100	4 Cont.	20	30	30	65
A-13	15 Days	Diesel Oil	100	4 Cont.	5	5	15	50
A-13	15 Days	Diesel Oil	100	30 Sec.	30	30	40	65
A-14	22 Days	Diesel Oil	100	30 Sec.	0	0	10	30
A-14	22 Days	Diesel Oil	100	60 Sec.	0	0	10	30
A-15	29 Days	Diesel Oil	100	60 Sec.	5	5	10	20
Screens weathered inside								
E-1	1 Day	Diesel Oil	100	4 Cont.	80	100	100	100
E-2	8 Days	Diesel Oil	100	4 Cont.	75	85	100	100
E-3	15 Days	Diesel Oil	100	4 Cont.	60	80	100	100
E-4	22 Days	Diesel Oil	100	4 Cont.	50	65	85	95
E-5	29 Days	Diesel Oil	100	4 Cont.	40	50	75	85
E-6	36 Days	Diesel Oil	100	4 Cont.	20	30	55	60
E-7	43 Days	Diesel Oil	100	4 Cont.	5	10	25	45
E-8	50 Days	Diesel Oil	100	60 Sec.	75	85	85	85
E-9	57 Days	Diesel Oil	100	60 Sec.	10	20	30	40
E-10	64 Days	Diesel Oil	100	60 Sec.	10	25	35	40

It is obvious from the above presentation that DDT in Diesel oil #2, applied at rate of 100 mgs. per square foot, retains its killing capacity after 4 contacts for longer periods when weathered inside than when weathered outside. The retention of killing capacity after weathering 36 days inside is almost identical with that same capacity when weathered only 8 days outside.

graphs and tables. (Graphs A and 1A + B to 5A + B, inclusive, and tables 1-5, inclusive.) They portray:

1. Effects of various solvents employing a dosage of 200 mgs. DDT per square foot.

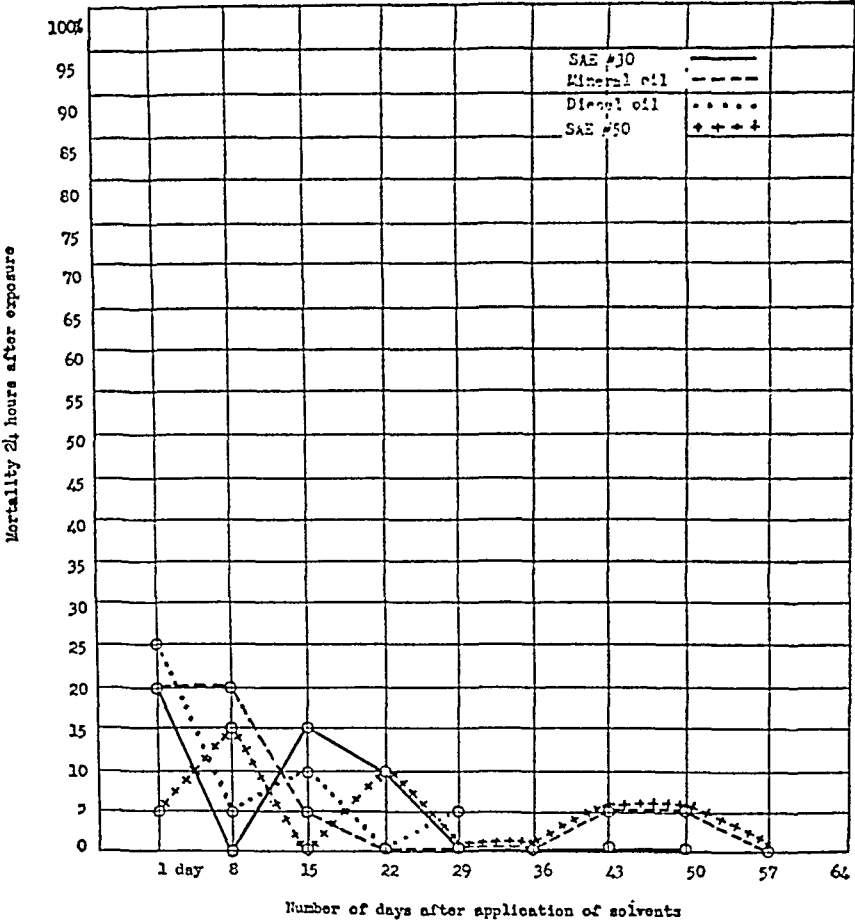
2. Effects of each solvent with varying dosage; namely, 200, 100, and 50 mgs. per square foot.

3. Comparison of "weathering" inside and outside of buildings. (During the period of "weathering," screens already treated with DDT were kept both inside and outside of the building.) (See Appendix No. 2.)

GENERAL CONCLUSIONS

(Note: It should be remembered that the mortality recorded, except as specifically noted, is after *Four Instantaneous Contacts* with DDT in solution.)

1. In preliminary experiments, mosquitoes were exposed to contact with DDT painted screen wire for as long as 30 seconds, but this relatively long period of

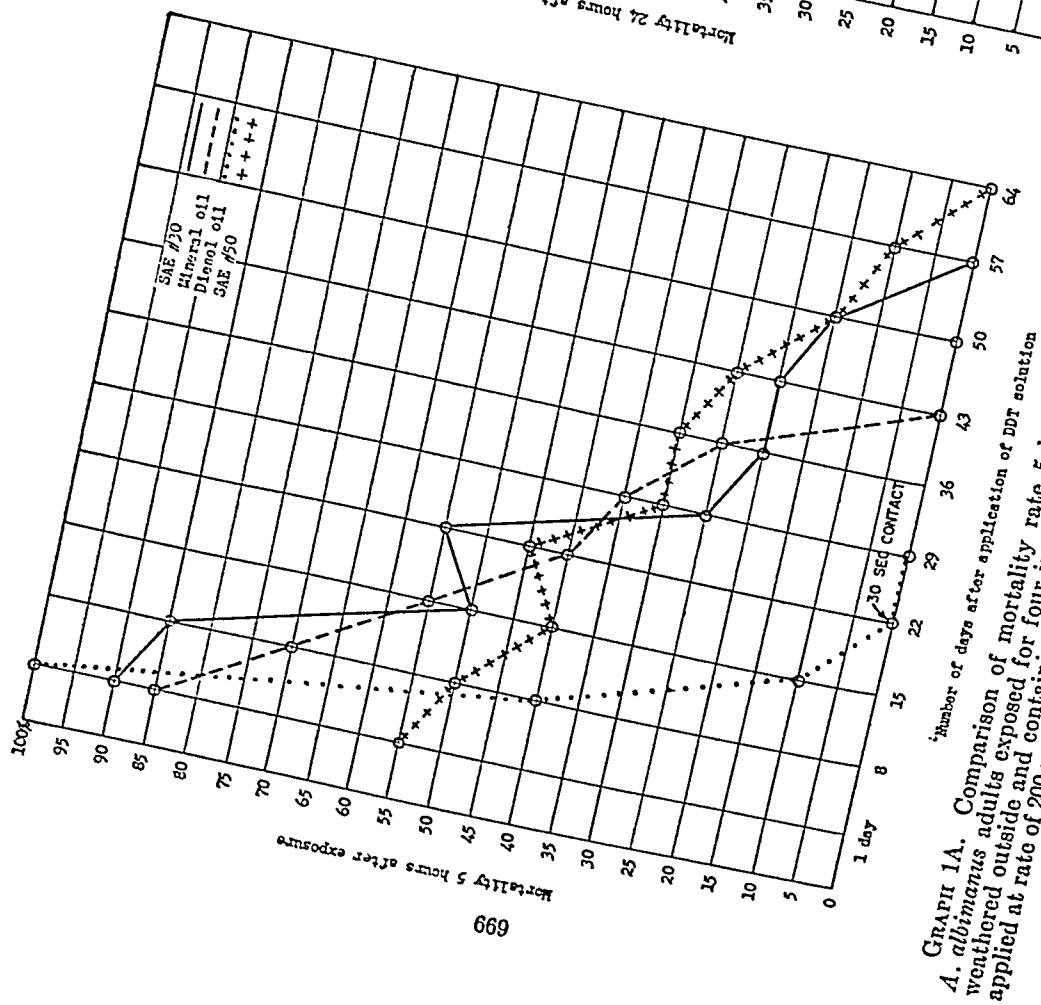


GRAPH A. Comparison of mortality rates after exposure of *A. albimanus* adults to four contacts with screens weathered for varying periods and previously painted with different solvents to which no DDT had been added.

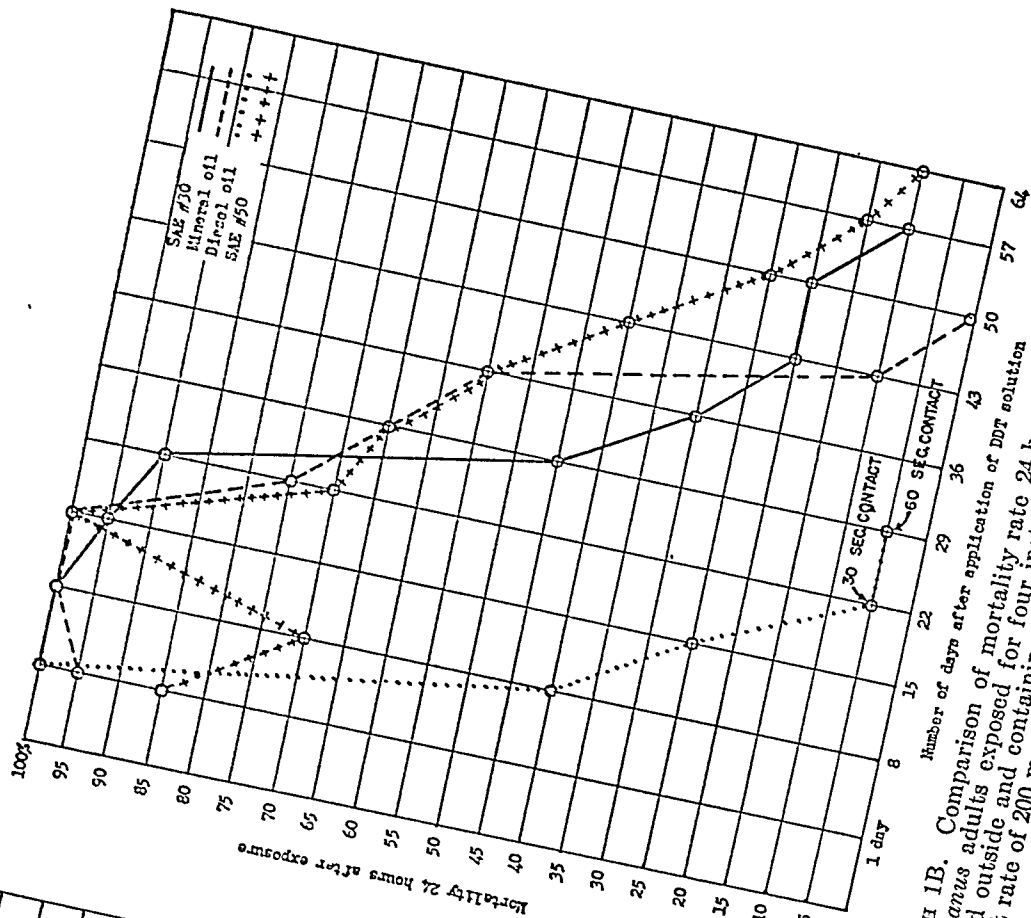
Note: No mortality 5 hours after exposure to Diesel oil, SAE #30 and #50. Only mineral oil showed a 5% mortality at the 5 hour period, and only on screens "weathered" one day.

exposure was soon found generally to be unnecessary if DDT was in solution. Accordingly, the present "contact exposure," much more severe than necessary for practical field comparison, was adopted.

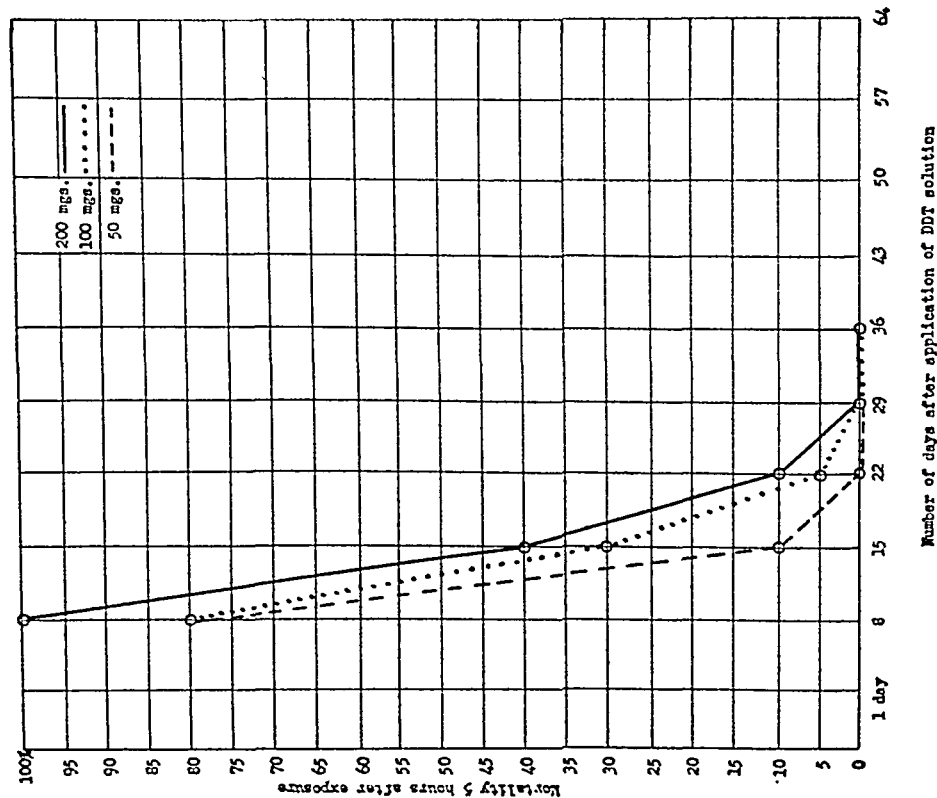
2. From the comparison Graph A and the "control" results recorded in the bottom line of tables 1-4, inclusive, (showing mortality of mosquitoes exposed to four contacts with various solvents and no DDT added, 5 and 24 hours after exposure), it is evident that there is a low mortality of mosquitoes in the comparison group caused evidently be exposure to the solvent with no DDT. That this



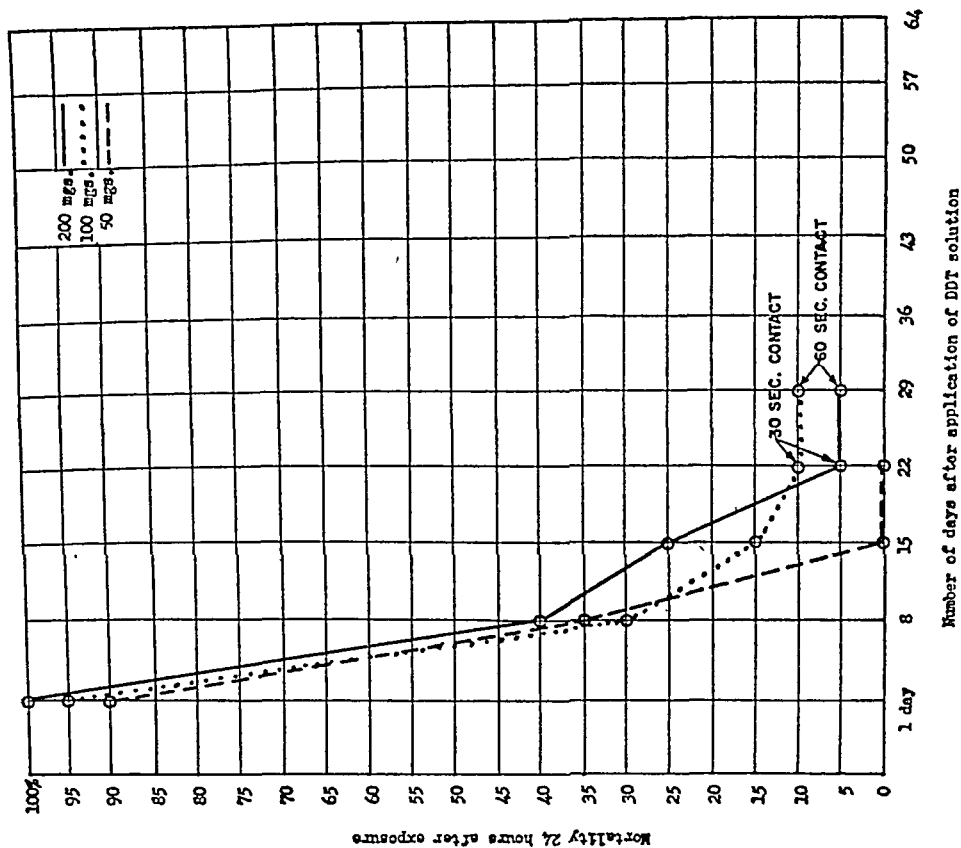
GRAPH 1A. Comparison of mortality rate 5 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in different solvents applied at rate of 200 mgs. per ft.<sup>2</sup>.



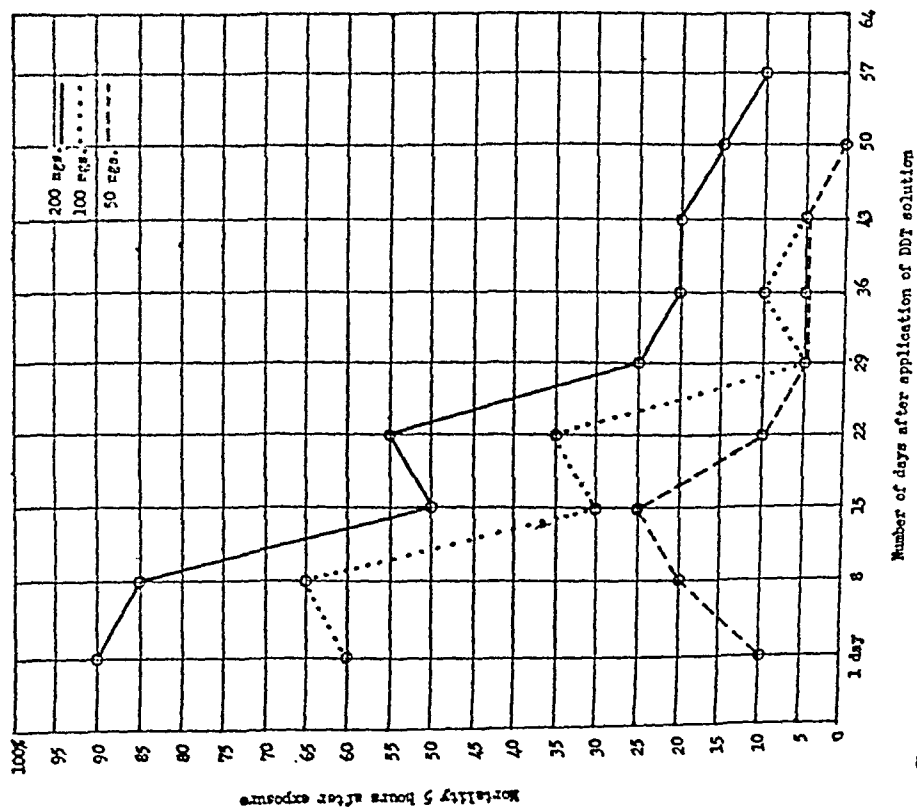
GRAPH 1B. Comparison of mortality rate 24 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in different solvents applied at rate of 200 mgs. per ft.<sup>2</sup>.



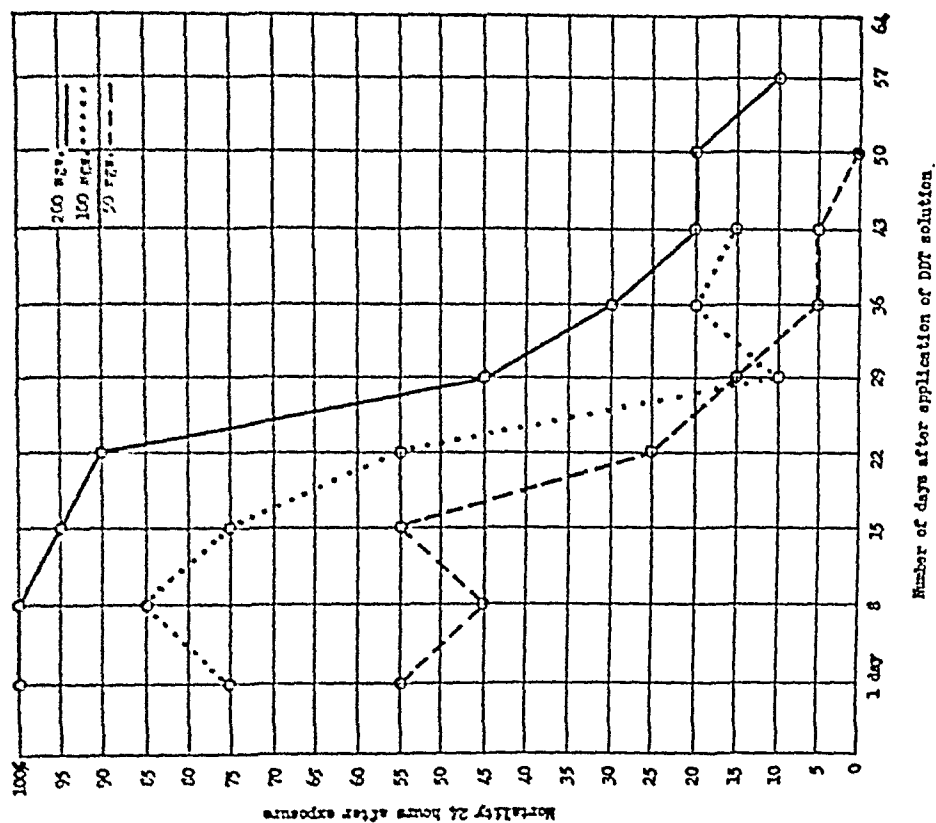
GRAPH 2A. Comparison of mortality rate 5 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in Diesel oil applied at rate of 200, 100, 50 mgs. per ft.<sup>2</sup>



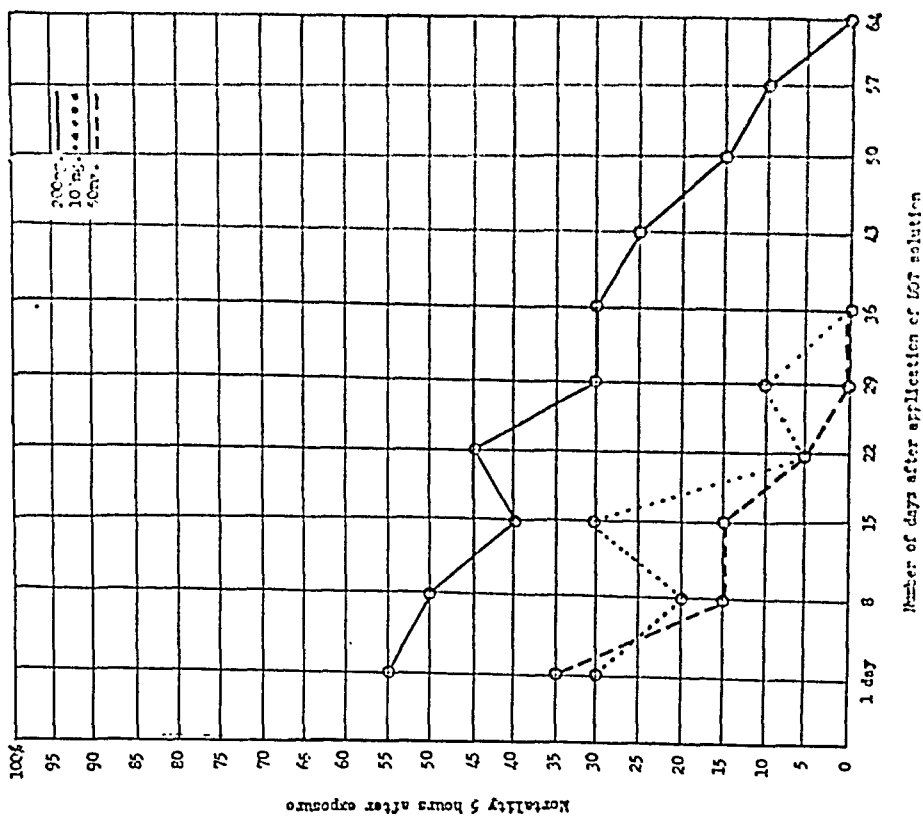
GRAPH 2B. Comparison of mortality rate 24 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in Diesel oil applied at rate of 200, 100, 50 mgs. per ft.<sup>2</sup>



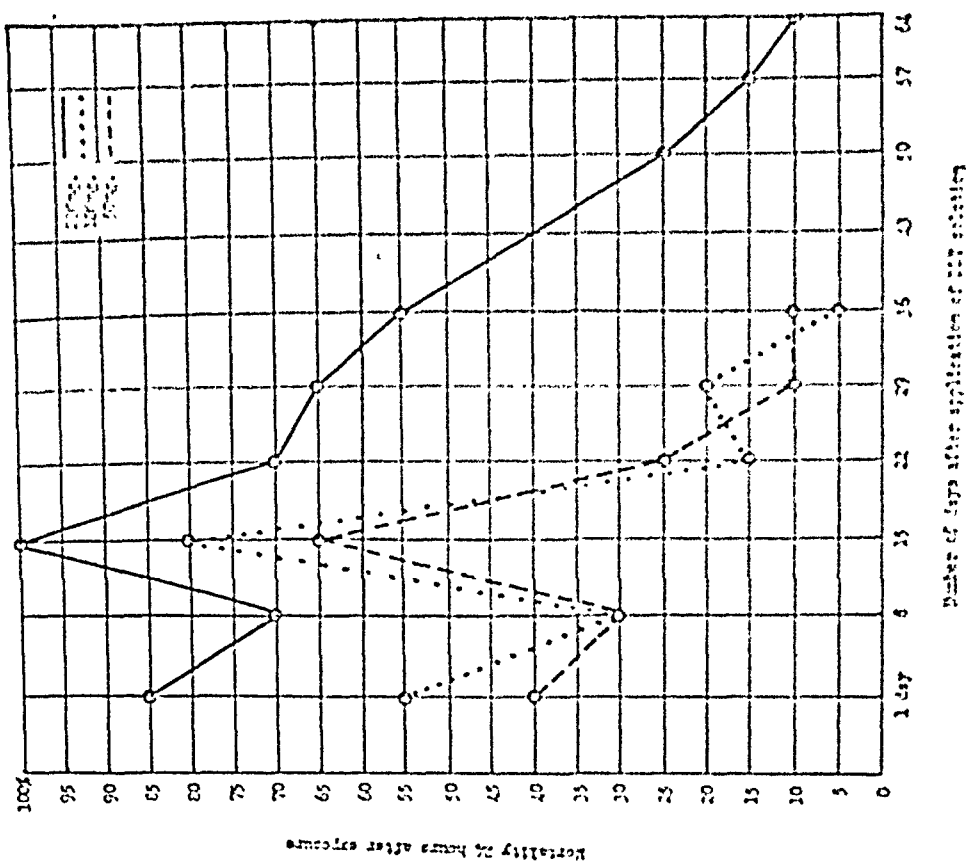
GRAPH 3A. Comparison of mortality rate 5 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in SAE #30 applied at rate of 200, 100, 50 mg. per ft.<sup>2</sup>.



GRAPH 3B. Comparison of mortality rate 24 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in SAE #30 applied at rate of 200, 100, 50 mg. per ft.<sup>2</sup>.

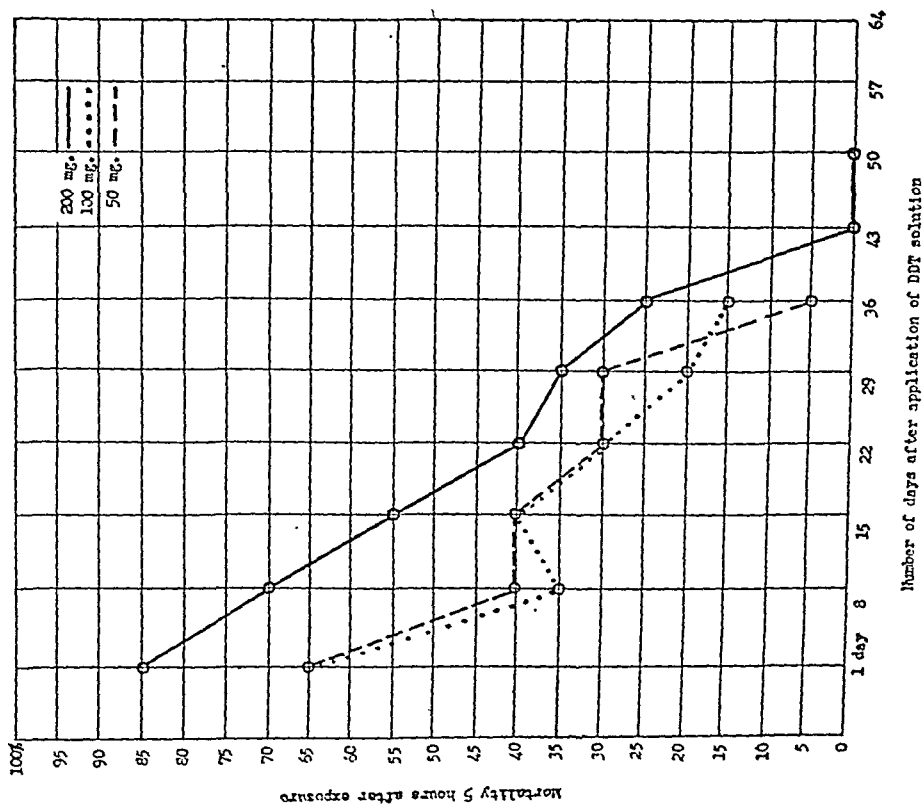


GRAPH 4A. Comparison of mortality rate 5 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in SAE #50 applied at rate of 200, 100, 50 mgs. per ft.<sup>2</sup>.

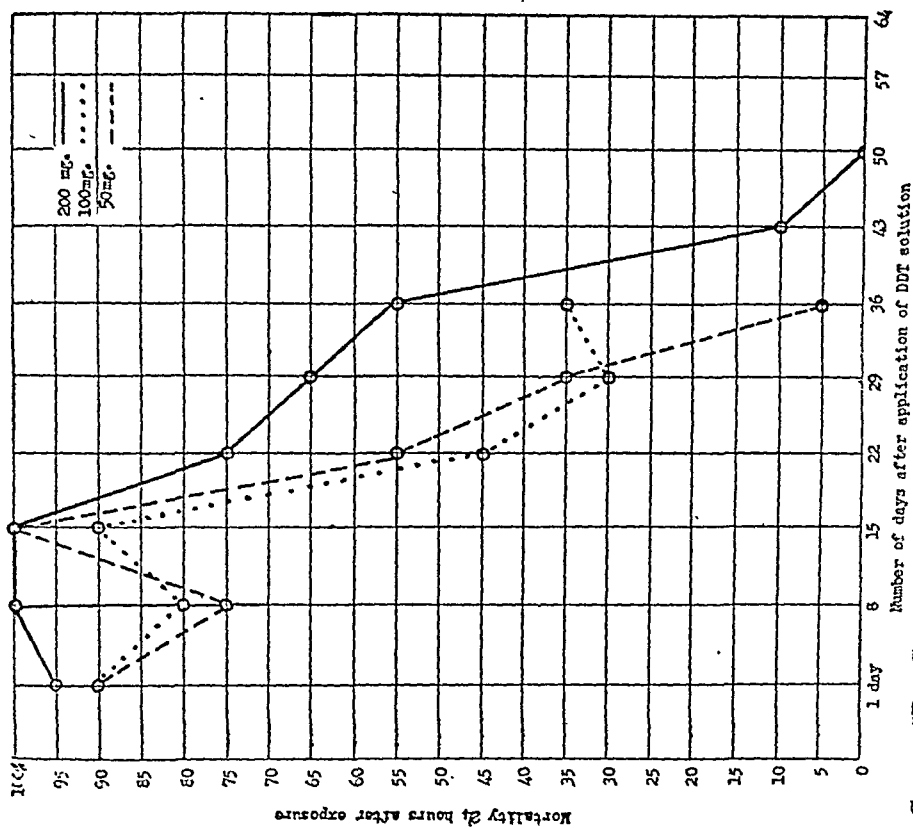


GRAPH 4B. Comparison of mortality rate 24 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in SAE #50 applied at rate of 200, 100, 50 mgs. per ft.<sup>2</sup>.





GRAPH 5A. Comparison of mortality rate 5 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in mineral oil applied at rate of 200, 100, 50 mgs. per ft.<sup>-2</sup>



GRAPH 5B. Comparison of mortality rate 24 hours after exposure of *A. albimanus* adults exposed for four instantaneous contacts to screens weathered outside and containing 5% DDT solution in mineral oil applied at rate of 200, 100, 50 mgs. per ft.<sup>-2</sup>

mortality is not due to the process of handling the mosquitoes (see Appendix No. 2) is evident from the fact that the tendency to mortality in the control group of mosquitoes decreases greatly or entirely disappears as the screens are "weathered" and the solvent evaporates or is removed by natural physical causes.

3. There is a wide disparity between the effect produced by Diesel oil and that produced by the other three solvents; namely, SAE #30 SAE #50, and mineral oil. The lethal effect of DDT in Diesel oil is excellent one day after the wire is

TABLE 6

*Showing rapid deterioration of lethal capacity of DDT in kerosene for adult mosquitoes with DDT applied to screens at rate of 100 and 200 mgs. per square foot, with various periods of contact, and at various ages of screens*

TEST DESIGNATION	AGE OF SCREEN	DOSAGE IN MGS. DDT	SOLVENT USED	PERIOD OF CONTACT ON DDT	% MORTALITY OVER VARIOUS PERIODS OF OBSERVATION			
					2 hrs.	5 hrs.	24 hrs.	48 hrs.
	hrs.							
Control*	24		Kerosene	4 contacts	0	0	0	0
Control†	24		Kerosene	30 seconds	0	0	20	30
No. 134	17	200	Kerosene	4 contacts	100	100	100	100
No. 138	19	100	Kerosene	4 contacts	100	100	100	100
No. 53	24	200	Kerosene	30 seconds	85	100	100	100
No. 54	24	200	Kerosene	20 seconds	60	75	75	80
No. 55	24	200	Kerosene	10 seconds	65	90	100	100
No. 117	25	200	Kerosene	4 contacts	45	50	80	85
No. 113	25	100	Kerosene	4 contacts	45	55	80	80
No. 118	25	200	Kerosene	10 seconds	40	55	85	90
No. 115	49	100	Kerosene	4 contacts	40	45	65	70
No. 119	49	200	Kerosene	4 contacts	0	0	5	5
No. 120	50	200	Kerosene	10 seconds	0	5	10	10
No. 57	71½	200	Kerosene	30 seconds	25	30	35	50
No. 58	91	200	Kerosene	30 seconds	0	5	15	20
No. 60	115	200	Kerosene	30 seconds	0	0	5	10

\* Control for experiments Nos. 113, 115, 117, 118, 119, 120, 134, 138.

† Control for experiments Nos. 53-55, 57-60.

treated, but this effect disappears rapidly on "weathering" outside; whereas, that quality persists in an effective manner after 29 days' "weathering" with the other solvents tested. Greater persistence of lethal effect was found with SAE #50 than with SAE #30, or mineral oil.

4. Diesel oil had the shortest persistence of action of all solvents here reported.

5. In previous tests (data not here presented in full) kerosene and acetone, as solvents, had a duration of action less than that for Diesel oil. (See table 6; also, Reactivation, table 7, Screens 84-88, inclusive.)

#### CONCLUSIONS—SPECIFIC

From a study of the facts portrayed in the graphs, the following points are evident:

## Section I

## Screens Weathered outside

SCREEN NO.	DOSAGE DDT PR. SQ. FT. MGS.	TIME ELAPSED FROM ORIGINAL PAINTING UNTIL LAST TEST BEFORE REACTIVATION		TIME ELAPSED SINCE REVITALIZATION OR RE-APPLICATION OF SOLVENT	PERIOD OF CONTACT DDT PAINTED SCREEN	PERCENTAGE OF MORTALITY AFTER EXPOSURE—MEASURED OVER VARYING TIME INTERVALS				SOLVENT	REMARKS
		Days	Hours			2 hrs.	5 hrs.	24 hrs.	48 hrs.		
A-5	200	29		60 Seconds		0	0	5	10	Diesel oil	Original Painting
A-6	200			None	4 Contacts	15	25	45	65	Diesel oil	Revitalized
A-7	200			7 Days	4 Contacts	0	0	20	55	Diesel oil	Revitalized
A-15	100	29		60 Seconds		5	5	10	20	Diesel oil	Original Painting
A-16	100			None	4 Contacts	5	15	20	30	Diesel oil	Revitalized
A-17	100			None	4 Contacts	5	15	20	30	Diesel oil	Revitalized
A-17	100			7 Days	4 Contacts	0	0	5	20	Diesel oil	Revitalized

## Section II

## Screens Weathered Inside

57	200		71½	30 Seconds	25	30	35	50	Kerosene	Original Painting
58	200		91	30 Seconds	0	5	15	20	Kerosene	Original Painting
60	200		115	30 Seconds	0	0	5	10	Kerosene	Original Painting
61	200		138½	30 Seconds	0	0	10	35	Kerosene	Original Painting
66	200			24 Hours 4 Contacts	85	90	100	100	Kerosene and Diesel oil	Revitalized
63	200			48 Hours 4 Contacts	30	30	35	35	Kerosene	Revitalized
67	200			48 Hours 4 Contacts	90	100	100	100	Kerosene and Diesel oil	Revitalized
64	200			72 Hours 4 Contacts	85	85	90	100	Kerosene and Diesel oil	Revitalized
68	200			72 Hours 4 Contacts	95	95	100	100	Kerosene and Diesel oil	Revitalized
65	200			96 Hours 4 Contacts	55	55	65	70	Kerosene	Revitalized
59	200			96 Hours 4 Contacts	80	90	90	100	Kerosene and Diesel oil	Revitalized
94	200	8		4 Contacts	0	0	0	0	Kerosene	Original Painting
84	200			24 Hours 4 Contacts	100	100	100	100	Kerosene	Revitalized
85	200			48 Hours 4 Contacts	70	80	85	85	Kerosene	Revitalized
86	200			72 Hours 4 Contacts	35	35	35	40	Kerosene	Revitalized
87	200			96 Hours 4 Contacts	30	45	45	55	Kerosene	Revitalized
88	200		24	120 Hours 4 Contacts	0	0	0	5	Kerosene	Revitalized
Control Exp. 94, 84-88				4 Contacts	0	0	0	0	Kerosene	Revitalized

1. Mortality after exposure increases, in general, with the increase of the period over which observations are made with the exception of Diesel oil. (See below.)
2. "Weathering" over sufficient time is associated with an inactivation of lethal effect of DDT to four contacts on the wire.
3. The lethal effect of DDT differs with different solvents.
  - a. Graph 1A and 1B. (Comparison of solvents with 200 mgs. of DDT per square foot with observations at the 5 and 24 hour periods.)
    - (1) Diesel oil produces the highest mortality on the first day after the painting when observations are made over the 5 hour observation period. At this early stage of "weathering" it acts more quickly than the other solvents tested.
    - (2) This 100% mortality is equalled by SAE #30 when observations of mortality among mosquitoes are recorded for the 24 hour period.
    - (3) The lethal quality of DDT, on four contacts only, rapidly disappears when in solution in Diesel oil, and remains effective over much longer periods with SAE #30 and #50 and mineral oil. SAE #50 and mineral oil effect 65% mortality during the 24 hour observation period after "weathering" 29 days, whereas the mortality produced by Diesel oil #2 drops from 100% at the 5 hour observation period after "weathering" 1 day, to 40% after "weathering" 8 days, and to 0% after "weathering" 22 days, when mortality again is observed over the 5 hour period.
4. Dosages of 200, 100, and 50 mgs. per square foot demonstrated in practically every period of observation, and with all solvents, that the higher dosage exceeded the lower in killing effect.
  - a. Exceptions
    - (1) Graph 2B. With Diesel oil and DDT, over the 24 hour observation period, mortality produced by the 200 mgs. screen fell below that of the 100 mgs. screen after 22 days of "weathering," and the 100 mgs. screen fell below the 50 mgs. screen on approximately the 10th day.
    - (2) Graph 3B. With SAE #30 and DDT, over the 24 hour observation period, the mortality produced by the 100 mgs. screen momentarily fell below that of the 50 mgs. screen after 29 days of "weathering," and then again exceeded the 50 mgs. screen on the 36th and 43rd day of "weathering."
    - (3) Graph 4A. With SAE #50 and DDT, over the 5 hour observation period, mortality produced by the 100 mgs. screen fell below that of the 50 mgs. screen after "weathering" 8 days, and remained below or equal on the 15th, 22nd, 29th, and 36th day of observation.
    - (4) Graph 4B. With SAE #50 and DDT, in the 24 hour observation period, the mortality produced by the 100 mgs. screen fell below that of the 50 mgs. screen on the observation made on the 22nd day of "weathering," but on the 29th day, the killing effect was again above that of the 50 mgs. screen.

- (5) *Graph 5A.* With mineral oil and DDT, at the 5 hour observation period, mortality produced by the 100 mgs. screen fell slightly below, or only equals, that of the 50 mgs. screen for the first 29 days of "weathering," and slightly exceeded the 50 mgs. screen after "weathering" 36 days.
- (6) *Graph 5B.* With mineral oil and DDT, 24 hours after exposure, mortality produced by the 50 mgs. screen exceeded that of the 100 mgs. screen, and equalled that of the 200 mgs. screen, on the observation made 15 days after "weathering." It fell below that of the 100 mgs. screen after 36 days of "weathering."
- (7) *Graph 2B.* In only one instance of 64 observations with the 200 mgs. dosage on screens over various periods of "weathering" did the lethal effect, as measured by mosquito mortality, fall below that of the 100 mgs. or 50 mgs. dosage. This one exception is shown in the Graph 2B, in which on the 22nd and 29th days of "weathering," the effect produced by the 200 mgs. screen fell slightly below that of the 100 mgs. screen.

At the time of painting the screens with DDT solution one set was prepared with Diesel oil No. 2 at the dosage of 100 mgs. of DDT per square foot. This set of screens, painted with one solvent only, and DDT, resting horizontally on rack inside the buildings, was tested against screens painted with the same dosage, and same solvent, and "weathered" on frames hung vertically outside windows of the building. The set was prepared to compare the effects of inside and outside "weathering."

Table 5 records the results of the studies. It is obvious from this study that DDT in Diesel oil No. 2, applied at the rate of 100 mgs. per square foot, retained its killing capacity after four contacts for longer periods when "weathered" inside of buildings than when "weathered" outside. The retention of killing capacity after "weathering" thirty-six days inside is almost identical with that of the screen "weathered" only eight days outside of the building.

(Note: In Test E-8, table 5, when the contact time on a screen "weathered" fifty days inside is increased from four contacts only to sixty seconds, the killing effect is greatly increased.)

#### ALLIED INVESTIGATIONS

Several allied investigations bear a relationship to the present set of experiments. This set of experiments, now to be reported, was accessory, and not planned to answer all related questions. They are here reported in a preliminary manner because of their relationship to the present subject.

These studies covered the following phases of investigation:

1. Exposure time necessary to produce mortality with *Aedes aegypti* resting on DDT in dry condition.
2. Reactivation of screen by reapplication of solvent after screen originally treated with DDT had lost its power to kill after only four contact exposures.
3. Inhibition of biting in mosquitoes that received four contact exposures to DDT.

EXPOSURE TIME NECESSARY TO PRODUCE MORTALITY WITH *Aedes aegypti*  
RESTING ON DDT IN DRY CONDITION

From the work performed, results of which are presented in the Graphs, it early became apparent that with dry DDT, after crystallization, mortality was not produced by an exposure as small as that of four contacts on screen wire. The painted screens gradually lost, over a period of time, their power to kill after this short contact period. Accordingly, experiments were performed to test killing capacity of DDT in dry crystalline form. Test-tubes were prepared with DDT crystals uniformly distributed over the inner surfaces at the dosage of approximately 200 mgs. per square foot. Groups of 20 *A. aegypti* were placed in the DDT-treated test-tubes, and allowed to rest on the inner surfaces for intervals of 2, 5, and 10 minutes, after which they were transferred from the tubes to a clean wire cage.

Twenty *Aedes aegypti* were placed in a similar wire cage, as a comparison group. Table 9 records the results of this experiment.

A second comparable experiment was performed utilizing *A. albimanus*, placed inside a copper wire screen cage 6" x 6" x 6", which had been previously treated with DDT solution.

The wire sides of the cage were treated with a 5% DDT solution, to leave a residue of approximately 200 mgs. of DDT per square foot. Two days after painting, the DDT had crystallized out of solution.

Twenty-five *A. albimanus* were then introduced into the treated cage.

17 minutes after exposure "knockdown" began.

22 minutes after exposure 70% were down.

27 minutes after exposure 100% were down.

From the first experiments here reported with *A. aegypti*, it is evident that DDT in its dry crystalline form killed 65% of *A. aegypti* making contact with it for two minutes, within the 16 hour observation period following contact. This mortality did not increase when mosquitoes were observed for 3 days after contact; whereas, after 5 minutes' exposure to the crystallized DDT, 100% of *A. aegypti* were dead inside of 2 hours. The kill on 5 minutes' exposure to dry DDT in above table is equalled by Screen No. 84, table 7, Section 11, where 100% mortality is recorded 2 hours after the mosquitoes had been exposed to 4 contacts of DDT in kerosene on a 24-hour old screen.

When in solution with various solvents (see Graph 1A) the following results were obtained after four instantaneous contacts:

Solvents	Mortality during 5 hour period after exposure (four contacts)
Diesel oil.....	100%
SAE #30.....	90%
Mineral oil.....	85%
SAE #50.....	55%

To produce results with exposure to the dry crystalline form of DDT, comparable to the four instantaneous contacts while in solution in varying solvents, a period of constant contact somewhere between two and five minutes was necessary. (*A. albimanus* used with DDT in solution, *A. aegypti* with dry DDT.)

REACTIVATION OF SCREEN BY REAPPLICATION OF SOLVENT AFTER  
SCREEN ORIGINALLY TREATED WITH DDT HAD LOST ITS POWER  
TO KILL AFTER ONLY FOUR CONTACT EXPOSURES

Soon after these investigations were begun, it became evident through the practice of minimal exposure to DDT, as made possible by the "four contact" technique, that the effect of DDT as a killing agent differed with different solvents. (*A. aegypti* and *A. albimanus* used as test mosquitoes.)

The screens treated with DDT in acetone (data not presented here in full) quickly lost their killing capacity, using four instantaneous contacts as a dosage. Kerosene retained this lethal capacity for a slightly longer period. This difference, however, amounted to a matter of hours only. With less volatile solvents the initial killing power was retained for much longer periods. It was, accordingly, of interest to ascertain from the academic, as well as the practical standpoint, whether the killing effect could be reactivated by re-dissolving in a solvent the DDT crystals which had crystallized because of the evaporation of the solvent.

As many pieces of screen wire had been painted with DDT in solvents on the same day, to be held for future testing of duration of action, material was at hand for testing reactivation of crystals of DDT on the screen wire, the solvent having evaporated. Reactivation tests were performed on samples of screen wire which had been preserved inside the building, as well as on those "weathered" outside.

Table 7 records the results of these tests.

Screens Nos. 57-61 and 63-68 in Section II of table 7 were all originally painted at the same time with 5% DDT in kerosene at rate of 200 mgs. per square foot, and were "weathered" inside the building. The first four tests recorded, 57, 58, 60, and 61, indicate the loss of killing power on four contacts, 71½ hours to 138½ hours after original painting.

Screens were revitalized by spraying some with kerosene, and some with kerosene and Diesel oil mixture, utilizing a quantity of solvent sufficient to cover the screen, but not to cause dropping of the solvent. A De Vilbiss nasal atomizer was used for the spraying, and each screen received a total of approximately 4.0 cc. of solvent, or of the mixture of the two solvents. Screens Nos. 59 and 63-68, inclusive, received such treatment with the solvent, as noted in the next to the last column of table 7. A second series of tests was performed with screens originally treated on the same day with 5% DDT in kerosene at rate of 200 mgs. per square foot (Screen Nos. 94 and 84-88) and all revitalized by spraying with kerosene.

Screen No. 94, table 7, shows the killing effect of the originally painted screens 8 days after they were painted. All killing power had disappeared for four

contact exposures. over an observation period of 48 hours. Screens Nos. 84-88, inclusive, show the same series of screens originally painted on the same day, then revitalized with kerosene, and their killing power recorded over the successive five days.

In these two series of experiments. Screen Nos. 63-68 and 84-88, it would appear that screens revitalized by application of the mixture of kerosene and Diesel oil produced longer duration of killing power than did kerosene when used alone.

The results recorded in table 7 demonstrate that screens can be revitalized so as to again possess killing power on short contact periods by treating with a solvent only, and accordingly, indicate that DDT in solution has a quicker killing effect on brief exposure, as found by using the "four contact" technique, than does DDT in crystallized form.

Screens 84-88, inclusive, also demonstrate the quick loss of killing power when kerosene is used alone as the solvent. From 100% kill in the 2 hour observation period the first day after revitalization, the capacity decreased to practically zero on the 5th day. This quick loss of potency corresponds to our experiments performed with kerosene as solvent, and not fully recorded here, except in tabe 6 and above in Screens 94 and 84-88, table 7.

Section I, table 7, shows the results of revitalization, when practiced on screens which have been "weathered" outside of the building as described in Appendix No. 2.

Screens Nos. A5 and A15 demonstrated the loss of killing power after 29 days' "weathering," with sixty seconds' exposure of the mosquitoes to screens originally treated with 200 mgs. per square foot of 5% DDT in Diesel oil No. 2.

Screens Nos. A6, A7, A16, and A17 show the results of revitalization on the "weathered" screens recorded on the day of revitalization, and seven days thereafter, using the "four contact" technique.

It is unfortunate that the reactivated screens outside had "weathered" for a longer period than those reactivated screens which had "weathered" inside, so rendering impossible exact comparison of reactivation after outside and inside influences.

In view of the rapid deterioration of killing power of the screens "weathered" outside, as compared to those "weathered" inside (see Table 5), it would appear that certain intrinsic factors, such as rain, wind, sunshine, and interaction of copper wire with DDT might be responsible. In view of the general experience with DDT, sunshine would appear to be the most probable deteriorating factor, and such possible deteriorating effect should be investigated in more detail.

#### INHIBITION OF BITING IN MOSQUITOES THAT HAVE RECEIVED FOUR CONTACT EXPOSURES TO DDT

Twenty-six separate tests were performed, each test using 20 *A. aegypti* mosquitoes. In addition to these 26 tests, 9 controls of 20 mosquitoes each were employed to correspond to each series of tests which were made on different days. Due to a plentiful supply, *A. aegypti* mosquitoes were used throughout in



test and control. These mosquitoes varied in age from 4 to 13 days. The DDT in Diesel oil was applied to the screens at the rate of 200 mgs. per square foot.

After being exposed to four contacts on the DDT in Diesel oil, painted on the screen wire 17½–23 hours previously, the mosquitoes were subsequently tested for biting, as compared to mosquitoes of the control group, which were handled

TABLE 8

*Summarizing inhibition of biting of A. aegypti by time intervals after exposure to four instantaneous contacts on screen wire painted 17½–23 hours before testing and treated with DDT in Diesel oil at rate of 200 mgs. per square foot*

NO. OF TESTS PERFORMED	NO. OF MOSQUITOES USED IN TEST	NO. OF MOSQUITOES BITING	% MOSQUITOES BITING	NO. OF ENGORGEMENTS	AVERAGE TIME (SECONDS) USED IN BITING	SUBSEQUENT PER CENT OF MORTALITY IN EACH GROUP OVER DIFFERENT TIME INTERVALS OF OBSERVATION			INHIBITION OF BITING RATE
						2 hrs.	5 hrs.	24 hrs.	
Control series									
9	180	176	97.7	176	12.9	0.55	0.55	1.6	2.2
Ten minutes after exposure									
6	120	85	70.8	85	17.4	58.3	70.0	80.8	29.2
Twenty minutes after exposure									
11	220	111	50.4	106	24.4	65.4	76.3	88.6	49.5
Thirty minutes after exposure									
9	180	70	38.8	68	25.1	58.8	73.3	89.4	61.1

TABLE 9

TIME EXPOSED TO DRY DDT	NO. DEAD AFTER 1 HOUR	NO. DEAD AFTER 2 HOURS	NO. DEAD AFTER 3 HOURS	NO. DEAD AFTER 4 HOURS	NO. DEAD AFTER 16 HOURS	NO. DEAD AFTER 24 HOURS	NO. DEAD AFTER 48 HOURS	NO. DEAD AFTER 72 HOURS
2 Min.	2 (10%)	7 (35%)	8 (40%)	10 (50%)	13 (65%)	13 (65%)	13 (65%)	13 (65%)
5 Min.	14 (70%)	20 (100%)						
10 Min.	14 (70%)	20 (100%)						
Control	0	0	0	0	0	0	0	0

in an identical manner but were exposed to four contacts on wire painted with Diesel oil alone.

Mosquitoes not biting within a two-minute interval, after being offered the forearm to bite, were considered to be inhibited to biting. These tests for biting ability were made on three groups of mosquitoes, after the lapse of 10, 20, and 30 minute intervals subsequent to exposure to DDT, one group for each time interval. Each mosquito in its original test-tube was offered the untreated and uncovered under surface of the forearm to bite. The results of these tests are summarized in accordance with each time interval in Table 8.

## CONCLUSIONS OF INHIBITION TEST

1. In the control series of 9 tests, 180 mosquitoes used, it is clear that the average of biting and resulting engorgements was almost perfect.

2. In the mosquitoes tested 10, 20, and 30 minutes after exposure to four contacts with DDT, there is a progressive decrease in the percent of those attempting to bite. In the group attempting biting, those becoming engorged practically equalled the number biting.

3. There is a corresponding increase in the percentage refusing to bite. Thirty minutes after exposure to four contacts with DDT, 61.1% did not bite.

4. Actual difficulty in biting, in those attempting to do so, rather than termination of desire alone, is apparent in the increasing time required to become engorged. As the time after exposure to DDT in solution increased, the time employed to accomplish engorgement increased from an average of 12.9 in the control group to 25.1 seconds in those tested 30 minutes after exposure.

5. The percentage of mosquitoes dead 2, 5, and 24 hours after exposure to DDT in Diesel oil is naturally comparable in all groups tested, as they were all originally exposed to DDT in solution for comparable periods. The mortality of 89.4%, 30 minutes after exposure, compares favorably with the 100% mortality after 5 hours recorded in Graph No. 1A.

6. Though the test insect used here was *A. aegypti*, it is reasonable to assume that anopheline mosquitoes infected with malaria parasites could bite and transmit malaria for a short time after exposure to four instantaneous contacts with DDT in Diesel oil at dosage of 200 mgs. per square foot. This ability to bite rapidly decreased over the 30 minute period after exposure to DDT in Diesel oil.

PRACTICAL APPLICATION OF EXPERIMENTAL DATA TO FIELD  
APPLICATION OF DDT FOR RESIDUAL KILL

1. DDT in solution proved more potent in its killing effect as measured by short contact periods, than DDT in dry crystalline form.

2. Differing solvents differed in their capacity to retain killing effect of DDT on short contact.

3. Two hundred milligrams of DDT per square foot in solution on screen wire proved to be more effective for kill on short contact than 100 or 50 mgs. per square foot.

4. Screen wire treated with DDT was revitalized by application of a solvent. This revitalization was more pronounced with screens "weathered" inside than those "weathered" outside. (Note: It may prove possible to revitalize inside walls, previously treated with DDT, simply by reapplication of a solvent with no DDT added.)

5. A percentage of mosquitoes, *A. aegypti*, exposed to four contact exposures of DDT in certain solvents was capable of biting man for a certain time after such exposure. Thirty minutes after such exposure, the percentage of mosquitoes unable to bite was large, reaching 61.1%. Accordingly, it is reasonable to assume that aerosol sprays, containing DDT alone, should not be relied upon to kill infected anophelines, if the building is to be entered immediately after such spraying.

6. These experiments showed an increase of mortality rates of mosquitoes exposed to dry crystallized DDT, increasing with time of exposure until the minimal lethal exposure time for 100% kill was reached. When in solution, with the solvents tested, and when the interval of time since the original application of DDT to the screen was not too long, four contacts<sup>6</sup> were usually sufficient to produce practically 100% mortality twenty-four hours after such contact. Such contact constituted a minimal lethal exposure. In the dry crystallized form this minimal period fell between 2 and 5 minutes of exposure.

7. The "four contact" exposure is a much more severe criterion of killing capacity than would normally exist under field conditions with residual DDT. Domestic species of anophelines in search of a human blood meal would presumably rest a considerable time on surfaces painted with DDT.

8. Kerosene and also Diesel oil #2 would appear to be less effective as practical solvents of DDT under most field conditions, than the other solvents tested, because of the relatively short duration of time over which DDT was held in solution with these solvents.

9. DDT in solvents, protected from the elements inside of buildings, retained its killing power against mosquitoes longer than when exposed outside of buildings.

## APPENDIX NO. 1

### FACTORS ASSOCIATED WITH TESTS

#### 1. Insects

Practically all tests were performed with *A. aegypti* and *A. albimanus* reared in the insectary of the Army School of Malariology.

#### 2. Materials

Copper wire (Army Issue) was used in all instances; all procured from one large piece reserved for the purpose.

DDT was commercial brand Army Issue.

Kerosene, Diesel oil #2, SAE oil #30 and #50 were all Army Issue.

Heavy American Mineral oil was the product of Louphil Laboratory, Inc., New York.

## APPENDIX NO. 2

### DETAIL OF PROCEDURE OF TEST

A central rectangular space 3" x 4" of pieces of copper wire 18" mesh, measuring overall 6" x 4 $\frac{3}{4}$ ", was painted with solution of DDT in the following solvents:

1. Diesel oil #2.
2. Lubricating oil SAE #30.
3. Lubricating oil SAE #50.
4. Mineral oil.

Quantities of the DDT in varying solvents were painted on the wire to repre-

<sup>6</sup> Unpublished findings indicate that 1, 2, and 3 contacts is not sufficient exposure to produce comparable results.

sent approximately 200 mgs., 100 mgs., and 50 mgs. per square foot. This dosage was determined by making solutions in the following manner:

5% solution in all solvents were used for the 200 mgs. series and 0.35 cc. of each solution was daubed on the screen by cotton on applicator sticks. (Cotton on applicator sticks was saturated with the solution before the process of application and left saturated on termination of the process.)

The solutions were prepared by dissolving 500 mgs. of DDT in 10 cc. of solvent.

200 mgs. of this solution would be contained in 40% (4 cc.) of the total amount, representing the quantity necessary for 1 square foot of surface.



FIG. 1. Showing procedure of application of mosquitoes to arm, making contact with screen wire painted with DDT solution.

As the surface of the wire actually painted, measured 3" x 4" or 12 square inches, one-twelfth of a square foot, 0.33 cc. would be indicated for such a surface. The amount actually used was 0.35 cc. as measurement of this amount proved to be easier in practice. To obtain the correct amount of DDT to represent 100 mgs. per square foot, 0.35 cc. of a 2.5% solution was used and to obtain an amount equivalent to 50 mgs. per square foot, 0.35 cc. of a 1.25% solution was used.

The number of screens (6" x 4 $\frac{3}{4}$ ") necessary for original and successive weekly testing was calculated for all dilutions of each solvent, and for all accessory tests. All dilutions of two solvents were painted on the respective screens on the same day, and all dilutions of the remaining two solvents on the following day. All screens were numbered for identification relative to solvent employed and dilutions; namely, at rate of 200, 100, and 50 mgs. per square foot.

At the same time of the painting of the screens with DDT solution, one set of screens to correspond to each weekly test was painted with the corresponding solvents along with no DDT added. Also, one set of screens with 100 mgs. of DDT per square foot in Diesel oil was painted in order to compare "weathering" effects on DDT solution inside of a building with "weathering" outside of the building. The individual pieces of processed wire were attached to wooden screen frames. These frames were placed on the outside of the laboratory building where they were exposed to the meteorological influences common to Panama during the period of April 26th to May 31st (Namely, the end of the dry season, and the beginning of the wet season). Twenty-four hours after painting the original screens, tests were made before the screens had been exposed to the weather, in order to determine original killing effect.

Tests on all solvents with DDT in solution at rate of 200, 100, and 50 mgs. were made at weekly intervals after "weathering," namely, on the 1st, 8th, 15th, 22nd, etc. day intervals. At the running of each weekly test, a test with solvent used on the screen with no DDT added was performed as well.

For the procedure of the test the appropriate "weathered" wire was adapted to the curve of the forearm with surface originally painted toward the outside. Over this was adapted a cardboard guard with 20 perforated holes of a diameter slightly less than the diameter of test-tubes used in test (see fig. 1).

The mosquitoes used in the tests were laboratory reared *A. albimanus*, all 4-7 days of age, and all of which had fed only on syrup and water.

Forty mosquitoes were introduced into test-tubes at the same time, 20 of those to be used in testing with the DDT solutions and 20 to be exposed to the action of solvent only.

The test-tube, with the original plug of *dampened* cotton removed, was then inverted over the cardboard at a point corresponding to hole No. 1. As soon as the mosquito had landed on the wire to bite, a piece of cardboard, comparable to a calling card, was inserted under the mouth of the test-tube between the cardboard cover and the test-tube with the result the mosquito was forced to break contact with the painted wire and fly upward in the inverted test-tube. This instantaneous exposure to the DDT painted wire constituted "one contact." Four such contacts, in attempt to secure a blood meal from the arm, were exacted of each mosquito. The time of each "contact" was recorded. For the second test the test-tube with mosquito inside was inverted over hole No. 2 of the cardboard guard and so on up to 20. The control group of mosquitoes were comparably exposed to contact with the wire painted with solvent, but no DDT. Observations on the mortality of the mosquitoes inside of the test-tubes were then made and recorded at 2, 5, 24, and 48 hour intervals.

(Note: The function of the cardboard guard placed on top of the painted wire was to prevent any DDT or solvent from sticking to the mouth of the inverted test-tube, and, by its possible later introduction into the tubes with the insertion of the moistened cotton stopper, causing exposure to DDT in excess of the "four contacts".)



though surveys depending on the submission of fecal samples require taking into account the possibility of substitution of samples by reluctant donors, we could detect, by repeat specimens and egg count data, only one instance in our series in which this occurred.

*Fecal examinations.*—In examining for helminthic ova our routine was as follows: Specimens were first displaced in dilution flasks, 4 ml. feces to 56 ml. water. (One ml. of feces was regarded as the equivalent of one gram. Stoll and Hausheer, 1926.) After the contents were shaken with glass beads, the flasks were refrigerated overnight and comminution completed the following morning. Ten ml. of fecal suspension, representing  $\frac{2}{3}$  gm. of the original feces, were put through the regular Lane floatation technic, using a four-bucket Lane (Turner) centrifuge. If the D.C.F. cover preparation was positive, the contents of the flask were made into decinormal sodium hydroxide suspension by the addition of one-hundredth the volume of 40 per cent NaOH (approximately 10/N NaOH). This addition of a minute amount of strong sodium hydroxide did not disturb the volumetric relationships significantly, and had the advantage of permitting dilution counts on the same fecal suspension originally sampled by floatation. The small drop (0.075 ml.) was used.

*Worm counts.*—When worm counts were desired, the men were admitted as patients to the Wards of Research Unit No. 2. Tetrachlorethylene was the drug of choice, and 4 ml. was the preferred dose. On the evening before treatment, supper was withheld and a preliminary purge of magnesium sulphate or sodium sulphate was given. A similar purge followed an hour after the anthelmintic in the morning. All stools passed for 24 hours were transported in bedpans to the laboratory. Worms were recovered by the gentle washing of the specimen under a simple shower spray through nested screens of 10, 20 and 40 meshes to the inch. The cleaned screenings were rinsed into large culture dishes for examination.

It is recognized that after treatment some worms continue to be expelled for 2, 3 or more days. The adoption of a 24-hour collection period gave, however, a serviceable comparative series, and probably accounted for over 90 per cent of the worms discharged after treatment.

*Units examined.*—Group I comprised five garrison-force organizations totaling 3270 men of whom 1241 or 38 percent were examined. They were:

A. 56th Naval Construction Battalion. This unit had been on Guam since August 1944, its only other Pacific duty having been 15 months on Oahu.

B. Naval Air Base. Duty on Guam was preceded by 3 to 4 months at Pearl Harbor. These men had been on Guam since August, 1944.

C. 9th Antiaircraft Artillery Battalion (Marine). Previous duty had been on Cuba, Guadalcanal, Rendova, New Georgia and Russell Islands before reaching Guam July–August, 1944.

D. Medical Research Unit No. 2. This unit arrived on Guam January 1945.

E. Naval Military Government Hospital No. 203 (Agana). Three-fourths of the staff personnel examined had arrived on Guam in September–November, 1944; the remainder arrived the succeeding three months.

Group II comprised three battalions of the 5th Amphibious Corps (Marine). Following a half-year in Hawaii, Group II participated in the Leyte campaign (artillery) and came to Guam in late December, 1944.

### RESULTS

*Incidence.*—Floatation, especially by the Lane technic, is held to be the most accurate method of demonstrating the presence or absence of hookworm eggs in feces. The incidence of hookworm found in the five garrison organizations of Group I and of the three battalions of Leyte-returned marines of Group II, is shown in table 1. It is apparent that as between each other, the suborganizations show no clear distinctions within the two groups. Altogether there were 71 positives in Group I, an incidence of 5.7 per cent, and 253 positives in Group II, or 34.1 per cent.

TABLE 1  
*Hookworm incidence in relation to organizations examined*

	GROUP I (GARRISON FORCES, GUAM)			GROUP II (LEYTE-RETURNED MARINES)		
	Per cent of roster examined	Number examined	Per cent positive	Per cent of roster examined	Number examined	Per cent positive
56 N.C.B.....	37	326	4.9			
N.A.B.....	25	184	6.5			
9th A.A.A.....	35	449	6.2			
NAMRU No. 2.....	51	142	3.5			
NMGH No. 203.....	87	140	7.1			
Hdq. Btry.....				37	99	20.0
5th G.B.....				39	251	43.0
11th G.B.....				57	392	31.9
Totals.....	38	1241	5.7	47	742	34.1
Officers only.....		43	0		41	34.1

When the data on the officers were examined separately, in Group I no positives were found among them; in Group II their rate was identical with that of the enlisted men.

The incidence data, examined in relation to age, period of enlistment and home States of the men, are shown in tables 2, 3 and 4. There is a slight but irregular trend toward a lower incidence with increased age (table 2) and with increased period in service (table 3) in both groups, but it is not marked. When home States are considered (table 4), there is clearly a loading in respect to men from the southern States, particularly those from the southern coastal States.

On the basis of these data concerning incidence alone, it is possible to conclude that in Group I we were dealing primarily with residual hookworm infections contracted at home, in Group II primarily with new infections acquired through exposure under combat conditions in the Philippines. The data may also indicate that hookworm-contracting habits (*i.e.*, soil pollution and walking barefoot),



TABLE 2  
*Hookworm incidence in relation to age*

AGES	GROUP I (GARRISON FORCES, GUAM)		GROUP II (LEYTE-RETURNED MARINES)	
	Number examined	Per cent positive	Number examined	Per cent positive
18-20	321	8.1	308	34.7
21-25	401	4.7	290	35.6
26-30	199	6.0	85	30.2
31 and over	275	4.0	41	25.8
Unknown	45	6.7	17	17.7
Totals.....	1241	5.7	742	34.1
Average age.....	Group I: 25.7 years		Group II: 22.6 years	

TABLE 3  
*Hookworm incidence in relation to period in service*

PERIOD IN SERVICE	GROUP I (GARRISON FORCES, GUAM)		GROUP II (LEYTE-RETURNED MARINES)	
	Number examined	Per cent positive	Number examined	Per cent positive
1st year.....	47	13.0	2	0
2nd year.....	469	5.1	394	37.1
3rd year.....	567	5.5	212	31.4
4th year.....	98	8.2	51	35.3
More than 4 years.....	42	4.8	42	28.6
Unknown.....	18	0	11	9.1
Totals.....	1241	5.7	742	34.1
Average period in service.....	Group I: 26.9 months		Group II: 27.0 months	

TABLE 4  
*Hookworm incidence in relation to home States*

HOME STATES	GROUP I (GARRISON FORCES, GUAM)		GROUP II (LEYTE-RETURNED MARINES)	
	Number examined	Per cent positive	Number examined	Per cent positive
*Southern coastal.....	178	20.8	85	48.2
†Other southern.....	321	4.7	127	37.0
Northern and western.....	714	2.7	523	31.6
Foreign and unknown.....	28	0	7	0
Totals.....	1241	5.7	742	34.1

\* Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi and Louisiana.

† Texas, Oklahoma, Arkansas, Missouri, Tennessee, Kentucky, West Virginia and Maryland.

TABLE 5

*Hookworm egg counts and 24-hour post-treatment worm counts; Group I, Garrison forces, Guam; Group II, Leyte-returned Marines*

GROUP	CASE, KEYED TO HOME STATE	HOOK-WORM EPG	24-HOUR POST-R <sub>x</sub> WORM COUNTS			AVERAGE WORM COUNT FOR EPG CLASS	EPG CLASS
			<i>Necator</i>	<i>Ancylostoma</i>	Total		
II	S-Penn	100	7	0	7	4	Less than 1000
I	LH-Ala	200	7	0	7		
I	G-Ark	300	6	0	6		
I	L-Tex	400	0	2	2		
I	P-Nebr	400	3	0	3		
I	E-Tex	600	0	3	3		
I	F-Calif	600	0	0	0		
II	L-Okla	700	3	4	7		
I	K-La	900	1	0	1		
I	L-N. Mex	1100	0	0	0	32	1100-5000
I	JM-Ark	1100	0	1	1		
I	T-La	1100	29	0	29		
II	E-N. J	1200	17	0	17		
I	CH-Ala	1400	21	0	21		
II	B-Ill	1800	0	4	4		
I	F-Tex	2100	71	0	71		
I	T-Miss	3000	0	5	5†		
I	B-La	4100	70	0	70		
I	B-Ala	4400	108	0	108		
II	B-N Car	5400	135	2	137	57	5100-10,000
II	V-Ohio	5700	2	24	27		
II	A-Tex	6800	39	2	41		
II	B-Wis*	7500	(7) 8	(11) 13	(18) 21		
II	T-Tex	8300	167	2	169		
II	S-Mo	8400	1	11	12		
II	S-Oreg*	8400	(2) 2	(11) 15	(13) 17		
II	LM-Ark*	8600	(5) 9	(17) 37	(22) 46		
I	P-Mich*	9600	0	(23) 47	(23) 47		
II	F-Nebr	10,200	358	13	371	211	Over 10,000 (13,000)
II	R-Mo*	10,200	(9) 9	(19) 22	(28) 31		
II	B-Mich	11,500	64	14	78		
II	E-Mich*	14,300	(77) 83	(69) 107	(146) 190		
II	J-Mass	19,000	373	15	388		
Total.....	33		1594	343	1937		
Per cent...			82.3	17.7	100.0		

\* The number of worms in these cases is from 2 treatments, with 24-hour collections after each; the results of the first treatment are given in parentheses.

† Known to have received treatment with chenopodium 6 weeks and 3 weeks previously.

In addition to the cases listed, there were 6 instances of men from whom one hookworm each (in 2 instances *Necator*, in 4 *Ancylostoma*) was recovered, although fecal examination by salt floatation had been negative.

*Egg counts and hemoglobin.*—Hookworm disease is related primarily to the anemia which results from the blood feeding of the adult worms in the intestine. Hemoglobin readings give, therefore, an objective measure of the essential damage due to the adult parasites. A comparison is thus possible between the worm burdens as measured by egg counts, and the anemia if present. Table 7 exhibits this relationship as determined on the 33 persons whose egg counts are shown in table 5, together with 176 members of the 5th G.B. (Group II) chosen at random for blood tests and egg counts. It is obvious immediately that this group of young adult Americans shows practically no indication of lowered hemoglobins despite increasing size of egg counts. The hemoglobin values in minimal positives are, if anything, higher than the negatives, while the hemoglobin average of

TABLE 6

*Severity of hookworm infection based on egg count classes (1241 men examined in Group I, 742 in Group II)*

SEVERITY OF INFECTIONS*	GROUP I (GARRISON FORCES, GUAM)			GROUP II (LEYTE-RETURNED MARINES)		
	Number positive	Per cent positive	Per cent of Group I	Number positive	Per cent positive	Per cent of Group II
Minimal.....	44	62.0	3.5	181	71.5	24.4
Light.....	22	31.0	1.8	56	22.1	7.5
Moderate.....	4	5.6	0.3	10	4.0	1.4
Heavy.....	1	1.4	0.1	6	2.4	0.8
Totals.....	71	100.0	5.7	253	100.0	34.1
Average EPG.....	Group I: 1580			Group II: 1400		

\* *Minimal*, less than 1000 EPG (eggs per gram); *light*, 1100-5000 EPG; *moderate*, 5100-10,000 EPG; *heavy*, over 10,000 EPG.

light, moderate and heavy positives is 98 per cent of negatives. These determinations were made approximately 6 months after the return of Group II to Guam, using the copper sulfate method of Phillips *et al.* (1943). It should be kept in mind that continued blood losses in persons with the heavier infections in another 6 months might register more specific damage.

*Egg counts and eosinophilia.*—Differential blood counts to determine the relationship of eosinophilia and hookworm egg counts in these men are presented in table 8 on 100 unselected individuals from Group II. While the two highest egg count cases show hypereosinophilia the correlation in the entire group is suggestive but not demonstrable. Special attention was paid to whether or not men with high eosinophilia who were hookworm negative showed evidence of schistosomiasis. No positive *Schistosoma japonicum* cases could be demonstrated, by fecal examination or symptoms. Nor was there any correlation between the eosinophilias and presence of *Ascaris* and *Trichuris*. In one instance, a man showing 35 per cent eosinophilia but negative for hookworm ova on stool examination was treated with anthelmintic and from him one male

*Ancylostoma duodenale* adult was secured. Non-egg-producing light infections with a few hookworms might thus have been responsible for an occasional eosinophilic leucocytosis.

*Necator americanus* and *Ancylostoma duodenale*.—The 33 cases listed in table 5 constitute our entire series from Groups I and II of worm counts on persons

TABLE 7  
Correlation of hookworm egg counts and hemoglobin values

EPG CLASSES	GRAMS HEMOGLOBIN PER 100 ML. WHOLE BLOOD									Total cases	Av. Hb.
	12.6 to 13.0	13.1 to 13.5	13.6 to 14.0	14.1 to 14.5	14.6 to 15.0	15.1 to 15.5	15.6 to 16.0	16.1 to 16.5	16.6 to 17.0		
Negative.....	1	2	8	15	26	39	13	8	1	113	15.0
Less than 1000..			5	5	14	12	15	5		56	15.2
1100-5000.....	2	0	4	2	6	6	5	1		26	14.8
5100-10,000.....		1	1	3	0	1	1	1		8	14.7
Over 10,000....				2	3	1				6	14.8
Totals.....	3	3	18	27	49	59	34	15	1	209	

TABLE 8  
Eosinophilia in Marines 6 months after return from Leyte campaign, in relation to hookworm egg counts

EPG	PER CENT EOSINOPHILIA											Total
	Neg.	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	
Neg.	2	21	11	7	1	3		1		1		47
1-1000	1	7	16	5	4	3		1				37
1001-2000			1	2	2	2	1	1				9
2001-3000			1			2				1		4
3001-4000												
4001-5000												
5001-6000				1								1
6001-7000												
7001-8000												
8001-9000												
9001-10,000												
10,001-11,000							1				1	2
Total.....	3	28	29	15	7	10	2	3	0	2	1	100

diagnosed as hookworm positives. Of these, 16 are from Group I (12 from 56 N.C.B., 1 from NAMRU No. 2, 3 from NMGH No. 203), and 17 are from Group II (12 from 5th G.B., 5 from 11th G.B.).

It is of profit to examine these worm counts in more detail. In Group I, no worms were recovered from 2 low egg count cases (F-Calif and L-N. Mex) in the first 24 hours after treatment. Five yielded only *Ancylostoma* (respectively 2, 3, 1, 5 and 47 from L-Tex, E-Tex, JM-Ark, T-Miss and P-Mich). These 5 are

considered evidence of infection on Guam, where 76 per cent of the hookworms recovered from natives after treatment or at autopsy have been *A. duodenale*, and 62 per cent of cases had only this worm (Stoll, 1946). The case of P-Mich is illuminating. In the service since March, 1944, on Guam since November, 1944, and 33 years of age, this man had duty in the hospital laundry. Due to conditions beyond control during rebuilding of the hospital, some soiled clothing and bed linen occasionally lay 7 to 10 days before being washed, and wet weather kept them damp. Thus, the appropriate moisture and temperature obtained which would allow development of infective larvae. In sorting the laundry later, this laundryman frequently noted itching in the skin of his hands, followed by episodes of marked throat irritation with unproductive cough, as well as gastrointestinal upsets. We are led to conclude that a special set of conditions here produced an occupational hookworm hazard, and, as the hospital patients were all Guamanians, it was predictable that his worms would be *A. duodenale*. This was the case as proved by a post-treatment worm count in August. Of 3 men assigned to this early laundry duty whose feces we examined, all 3 were positive for *Ancylostoma*.

The other 9 cases from Group I in table 5 showed pure *Necator americanus*, from 1 to 108 (average 35) per man. Eight of these men came from the southern States, and one (P-Nebr who had 3 *Necator*) had been in a camp in Texas. It is likely that these 9 men were carrying worm burdens contracted in the United States, as illustrated by the case of B-Ala. He had been nowhere in the Pacific except in camp at Pearl Harbor for 18 days while en route to Guam where he arrived in January, 1945. When treated in July, he had been in the NAMRU No. 2 unit 9 months and in the service 11. Nineteen years of age, his rural home was near Mobile, Alabama. He reported having had occasional treatments for "worms" in his boyhood. The 108 *N. americanus* found in his stools after treatment are thus ascribed to infection in Alabama.

The 17 Group II cases present a different picture. Omitting the instances in which only one hookworm was secured from each of 6 individuals who had been negative on fecal examination, there were only 2 (S-Penn and E-N. J) who had pure *Necator* infections of 7 and 17 worms, respectively, one (B-III) who had pure *Ancylostoma* (4 worms), but 14 who had both species. Eight of the 15 cases with *A. duodenale* were from northern States; the two largest worm counts (of 371 and 388 hookworms) were from men whose homes were Nebraska and Massachusetts. From the 17 Group II cases selected for worm count after treatment, with data in table 5, the average burden demonstrated was 92 hookworms, of which 17 or approximately a fifth, were *Ancylostoma*. The highest individual *Ancylostoma* count was 107 worms (E-Mich). This man was treated twice, in view of a large residual egg count after the first treatment.

It is worth emphasizing that 15 of 17, or 88 per cent, of the Group II cases which we studied by worm count harbored *Ancylostoma*. If this percentage is applied generally to the Leyte-returned marines who were hookworm positive, then 3 in every 10 marines (88 per cent of 34.1) had become infected with *A. duodenale*.

*Reinfection.*—If it could be shown that hookworm exposure occurred only during combat operations, as in the Philippines, the event would have less significance than if reinfection occurred under garrison conditions, as on Guam. Three instances of men from northern States who had not been west of Guam (Group I) and showing counts in the 5100–10,000 eggs per gram class, were considered probable *Ancylostoma* cases from their history. Unfortunately none of these three could be worm counted.

Besides the P-Mich case with 47 worms, already discussed, four other Group I cases listed in table 5 with *Ancylostoma* showed respectively 2, 3, 1 and 5 worms. These are small worm burdens unless life under garrison conditions permits reinfection. This we consider to be true, as judged by both indirect and direct evidence. On the indirect side, close questioning revealed that service men have little compunction against soil pollution when toilet facilities are not available. Also walking barefoot occurs out of bathing suits as well as in. During training programs, etc., auto mechanics working on muddy tires, signalmen and engineering squads working through muddy fields, obviously get effective contact with wet Guam soil. These factors can combine to result in reinfection, when considered in relation to the added hazard of soil pollution by natives.

Recently Loughlin and Stoll (1946) demonstrated an additional method by which service personnel could become infected with hookworm, namely by fomite-borne ancylostomiasis. It was shown that clothing and cotton blankets soiled with feces even lightly, and then kept moist for 5 days on Guam, allowed the development of infective larvae. These conditions could obtain under service conditions either by not changing clothing for a week, as under combat conditions, or by wearing clothing earlier soiled, which had been in a damp heap for a period of days.

During the course of our post-treatment worm counts, at least 3 marine cases furnished direct evidence that reinfection was occurring. This evidence was in the form of immature adult hookworms recovered after treatment. In each of 2 cases, about 1 per cent of the worms were young; in a third case, more than 10 per cent were young. These men were being treated more than 5 months after their return to Guam following about 2 months in the Philippines. These young adult hookworms were of a size indicating that the patients probably became infected about one month previously. Hookworm larvae acquired in Leyte would not account for these young worms, which instead are ascribed to infection on Guam in the interval after the marines returned to the Island. Both hookworms species were involved.

#### DISCUSSION

*Group I.*—The striking fact brought out in our study of shore-based Naval personnel who arrived on Guam after its American re-occupation in July–August 1944, and thus constituted a garrison rather than a combat force, is the small amount of hookworm demonstrated, approximately 1 man infected of 20. The preponderance of positive findings in men from the southern States, and the recovery of pure *Necator americanus* from many of them after treatment, indi-

worm incidence of 34.1 per cent. Again, many of the infections were light, but the percentage showing more than 5,000 eggs per gram of feces was 5 times more than that of Group I. From worm count data, it is estimated that 3 in 10 of the marines examined had *Ancylostoma*.

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# EXPERIMENTS TO DETERMINE POTENTIAL MOSQUITO VECTORS OF *WUCHERERIA BANCROFTI* IN THE CONTINENTAL UNITED STATES

## PART 2

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The study of the potential mosquito hosts of the periodic strain of *Wuchereria bancrofti* in the United States previously reported by Newton, Wright, and Pratt (1945)<sup>1</sup> has been continued. This report indicates the results obtained since the writing of the last paper.

A comprehensive survey of the literature on potential mosquito hosts occurring in the continental United States was made in the previous paper and need not be repeated. However, the work of Scott, Richards, and Seaman (1945) has been published since the previous report. These investigators, working in California, obtained infective larvae of *Wuchereria bancrofti* in *Culex erythrorhox*, *C. quinquefasciatus* and *C. tarsalis* which had fed upon a volunteer infected with the periodic strain of the filariid. Developmental stages of the larvae were found in 10 of 38 specimens of *Aedes taeniorhynchus* prior to 17 days, although the authors did not describe how far the development had progressed. Dissections of *Anopheles maculipennis* and *A. pseudopunctipennis* were negative. However, the number of dissections of these two species was inadequate.

The experimental methods employed in the work reported herein were identical with those described in the earlier report.

### EXPERIMENTAL FEEDING HABITS AND DISTRIBUTION OF SPECIES TESTED

The discussion of the feeding habits and distribution of species in this report has been limited to those species not included in the previous report. Distribution records have been taken for the most part from King, Bradley, and McNeel (1944) but have been supplemented from Matheson (1944) and from data compiled in this laboratory.

A total of 225 feedings was made between October 1944 and October 1945. In all, 9122 mosquitoes were used, of which 2087 partook of a blood meal (table 1).

*Anopheles crucians* fed fairly well in the lantern globes. This species is rather common along the coastal plains of the Southern United States and the

<sup>1</sup> The present studies are a continuation of those inaugurated at the School of Tropical Medicine, San Juan, Puerto Rico, and carried out through the generous cooperation of Dr. P. Morales Otero, Director, and Dr. J. Oliver-González, Head, Department of Medical Zoology. The authors are greatly indebted to these individuals for the facilities furnished and the active encouragement which they lent to the work. Thanks are due also to Dr. E. L. Bishop, Director of Health, Tennessee Valley Authority, for his kindness in providing laboratory space for further studies and for his interest in the project.



Gulf of Mexico. Its distribution, though spotty, extends along the Atlantic Coast to Massachusetts and westward into Southern Illinois. It was the predominating anopheline about Hattiesburg, Mississippi, during the winter months of 1944-1945.

*Anopheles maculipennis freeborni* fed exceedingly well under laboratory conditions. This species is common on the Pacific Slope. The material used in tests was obtained from a colony maintained at Columbia, South Carolina, by the Malaria Research Laboratory of the U. S. Public Health Service.

*Aedes canadensis* was an extremely poor feeder under the conditions employed during tests with this species in spite of its reputation for being very troublesome

TABLE 1  
Feeding data on mosquitoes exposed to blood meals from infected volunteers

SPECIES	NUMBER OF FEEDING ATTEMPTS	TOTAL NUMBER OF MOSQUITOES EXPOSED TO MEAL	TOTAL NUMBER OF MOSQUITOES FED	APPROXIMATE PER CENT FED
<i>Anopheles crucians</i> .....	16	854	171	20
<i>A. maculipennis freeborni</i> .....	16	422	223	53
<i>A. quadrimaculatus</i> .....	13	376	199	53
<i>Aedes canadensis</i> .....	25	2,287	89	4
<i>A. thibaulti</i> .....	13	1,111	166	15
<i>Culex erraticus</i> .....	30	618	66	11
<i>C. pipiens</i> .....	15	389	117	30
<i>C. restuans</i> .....	10	471	0	0
<i>C. salinarius</i> .....	34	769	166	22
<i>Mansonia perturbans</i> .....	12	407	134	33
<i>Orthopodomyia</i> sp.....	2	19	0	0
<i>Psorophora ciliata</i> .....	13	578	290	50
<i>P. cyanescens</i> .....	9	372	231	62
<i>P. discolor</i> .....	13	399	285	71
<i>P. ferox</i> .....	2	21	4	19
<i>P. howardii</i> .....	2	29	6	21
Total.....	225	9,122	2,087	

in the forests. This species is widely distributed throughout the United States, particularly in the northern half.

*Aedes thibaulti* fed fairly well. This species is distributed rather sparingly throughout the Southern States, breeding in stump holes. However, it occurred in large numbers in those spots where it was found.

*Culex pipiens* fed quite well when allowed to feed in large cages while the volunteer slept. This is the common house mosquito of Northern United States. The material used in tests was from a colony started from egg rafts supplied by the Army Medical School at Washington, D. C.

All efforts to feed specimens of *Culex restuans* were unsuccessful.

*Mansonia perturbans* fed quite well in lantern globes. Because of the difficulty of obtaining larvae of this species, it was necessary to feed wild adults

caught in the woods while attempting to bite. This species is widely distributed throughout North America.

A few specimens of *Orthopodomyia* sp. were obtained but none of these fed.

*Psorophora cyaneescens* fed very readily in the laboratory. This species is widely distributed throughout the Southern States.

TABLE 2  
Development of *W. bancrofti* larvae in various species of mosquitoes

SPECIES	EARLY DEVELOPMENT OF LARVAE—2 TO 9 DAYS AFTER FEEDING		LATE DEVELOPMENT OF LARVAE—9 DAYS OR MORE AFTER FEEDING				TOTAL INFECTIBILITY PERCENTAGE	NUMBERS OF INFECTIVE LARVAE PER SPECIMEN	
	Number of mosquitoes dissected	Number showing normal larvae	Number of mosquitoes dissected	Number showing well advanced, but not infective, larvae	Number showing infective larvae anywhere in body	Number showing infective larvae in head or proboscis		Maximum	Average
<i>Anopheles crucians</i> ....	22	11	126	2	1	0	2.4	1	1
<i>A. maculipennis freeborni</i> .....	25	0	105	0	0	0	0.0		
<i>A. quadrimaculatus</i> ....	20	0	116	0	0	0	0.0		
<i>Aedes canadensis</i> .....	10	0	59	0	0	0	0/59*		
<i>A. thibaulti</i> .....	13	1	108	3	0	0	2.8		
<i>Culex erraticus</i> .....	1	1	49	5	9	4	14/49	9	4
<i>C. pipiens</i> .....	5	4	108	14	84	64	91	53	14
<i>C. salinarius</i> .....	33	14	109	3	0	0	2.8		
<i>Psorophora ciliata</i> ....	15	0	108	0	0	0	0.0		
<i>P. cyaneescens</i> .....	3	1	51	0	0	0	0/51		
<i>P. discolor</i> .....	33	29	120	42	38	19	67	15	5
<i>P. ferox</i> .....	0	0	2	0	0	0	0/2		
<i>P. howardii</i> .....	0	0	4	0	0	0	0/4		
<i>Mansonia perturbans</i> ..	10	0	58	0	0	0	0/58		

\* Percentages not computed on less than 100 late dissections.

*Psorophora discolor* was found to be more abundant than was previously described in the former paper, occurring in appreciable numbers in almost every breeding area where other *Psorophora* were found.

A few *Psorophora ferox* were obtained and these did not feed nearly as well as this species does in the woods. This species is distributed rather widely throughout the United States although the larvae are difficult to find.

A few *Psorophora howardii* were encountered and these fed fairly well. This species occurs throughout the Southern States although it is not common.

#### LARVAL DEVELOPMENT

The development of the larvae within the mosquitoes followed the same pattern described by other workers and by Newton, Wright, and Pratt (1945) in the earlier report on this project. In susceptible species the infective stage

usually was reached within two weeks. However, 5 to 7 days additional time was required in *Psorophora discolor*.

Development was arrested at the first stage in those species in which it was incomplete or abortive. In some species the microfilariae never reached the thoracic musculature. This was the case in *Anopheles quadrimaculatus* and *A. maculipennis freeborni* in which dead and encapsulated microfilariae were found in the abdominal cavity 12 hours after the blood meal.

#### RESULTS OF EXAMINATIONS

A total of 1314 dissections was made of 14 species of mosquitoes. The results of these dissections are given in table 2. This table contains data on species not discussed in the previous report. In addition, it includes the final results, with the exception of *Culex erraticus*, obtained with species which had not been adequately tested at the time of writing the previous report. A discussion of the results obtained with each species is given below:

*Anopheles crucians*. A total of 148 specimens was examined of which 126 survived  $9\frac{1}{2}$  days or longer after an infected blood meal. Of the 126, one mosquito contained an infective larva and 2 other mosquitoes contained third-stage larvae at a late stage of development. The larvae often developed normally up to the sausage stage in this species. Half of the mosquitoes dissected before  $9\frac{1}{2}$  days contained early forms which appeared to be in good condition. However, in mosquitoes dissected at a later date the larvae usually had not developed beyond the first stage and were dead and often encapsulated. An infectibility rate of about 2 per cent was obtained with this species.

*Anopheles maculipennis freeborni*. A total of 130 mosquitoes was dissected, of which 105 had survived  $9\frac{1}{2}$  days or longer. With but few exceptions the specimens examined were negative. The larvae that were encountered were dead and degenerate first-stage forms. Some specimens examined within a few hours after feeding contained larvae that were becoming encapsulated in the abdominal cavity.

*Anopheles quadrimaculatus*. Studies were continued with this species. A total of 136 dissections was made, of which 116 were of mosquitoes surviving  $9\frac{1}{2}$  days or longer. By far the majority of these was negative and what few larvae were encountered were dead first stages. Early first-stage larvae were encapsulated in the abdominal cavity of this species within a few hours after feeding. The results obtained with this species closely approximated those obtained with *A. maculipennis freeborni* in every respect.

*Aedes canadensis*. Many hundreds of females of this species were exposed to blood meals but so few fed that a total of only 69 dissections was obtained. Of these, 59 were of mosquitoes which had survived  $9\frac{1}{2}$  days or longer. All dissections were entirely negative.

*Aedes thibaulti*. A total of 121 dissections was made, of which 108 had survived for  $9\frac{1}{2}$  days or longer. Of the latter, 3 mosquitoes contained normally developing late stages although no infective larvae were found. Only one of 13 mosquitoes dissected prior to  $9\frac{1}{2}$  days contained normal larvae. The ma-

jority of the specimens examined were completely negative, and with the above 4 exceptions what larvae were found had ceased development early in the first stage. A possible infectivity rate of 3 per cent was obtained with this species.

*Culex erraticus*. Study of this species was continued. A total of 50 dissections was made, of which 49 were of specimens surviving  $9\frac{1}{2}$  days or longer. Of the latter, 9 contained infective larvae and an additional 5 contained larvae at a late stage of development. The other 35 were negative. No incomplete or abortive development of the larvae was noted in this species. An infectibility rate of 14 per 49 was obtained.

*Culex pipiens*. A total of 113 dissections was made of this species. Of these, 108 were of mosquitoes surviving  $9\frac{1}{2}$  days or longer after feeding. In 84 of the 108 there were active infective larvae. Sixty-four of these mosquitoes had head or proboscis infections. Of the other 24 which survived  $9\frac{1}{2}$  days or more, 14 contained late stages of the larvae which had not attained the infective stage. Four of the 5 examined at an early date contained normal larvae. A total infectibility rate of 91 per cent was obtained with this species.

*Culex salinarius*. Study of this species was continued. A total of 142 dissections was completed. Of these, 109 were of mosquitoes which had survived  $9\frac{1}{2}$  days or longer after feeding. No infective larvae were recovered although third-stage larvae were found in three of the specimens. Of the 33 mosquitoes examined prior to  $9\frac{1}{2}$  days, 14 contained normally developing larvae. In about half of the dissections larvae were recovered. However, with the three exceptions mentioned, development had ceased at the presausage stage. This species had a potential infectibility rate of 3 per cent.

*Mansonia perturbans*. A total of 68 dissections was made of this species. Of these, 58 were of mosquitoes which had survived  $9\frac{1}{2}$  days or longer. None of the latter contained infective or late stages of the filariid. There was no normal development encountered in the 10 dissected prior to  $9\frac{1}{2}$  days. The majority of the specimens was negative and those larvae encountered were dead first stages.

*Psorophora ciliata*. A total of 123 dissections was made of this species, of which 108 were late dissections. No development beyond the first stage was found. The majority of the specimens examined was negative and most of the larvae found were dead and had been encapsulated at the early first stage. None of 15 early dissections contained normally developing larvae.

*Psorophora cyanescens*. A total of 54 dissections was made, of which 51 were of mosquitoes which had survived  $9\frac{1}{2}$  days or longer. In none of the 51 late dissections were there found late or infective larvae. One of three specimens dissected at an early date contained normally developing larvae. Over half of the mosquitoes examined contained larvae; but the latter had not developed beyond the presausage stage, and many were dead and undergoing degeneration.

*Psorophora discolor*. In all, 153 dissections were made of this species. A total of 120 of these dissections was made  $9\frac{1}{2}$  days or longer after feeding. A large proportion of the late dissections was made after  $18\frac{1}{2}$  days because of the abnormally long period of development required for the larvae to become in-

fective in this species. In the 120 late dissections a total of 38 specimens contained infective larvae, and half of these mosquitoes had proboscis or head infections. An additional 42 contained larvae in late stages of development. At the time of dissection these had not yet had sufficient time to reach the infective stage. Of the 33 examined prior to 9½ days, 29 contained normal larvae. On the basis of these results an infectibility rate of 67 per cent was obtained with this species.

*Psorophora ferox*. Only two of this species were examined, both of which had survived at least 9½ days. One mosquito was negative and the other contained 3 presausage larvae which were encapsulated.

*Psorophora howardii*. Only four individuals of this species were examined, all of which had survived 9½ days after feeding. All specimens were negative.

#### DISCUSSION OF RESULTS

The results obtained with *Culex pipiens* indicated that this species would in all probability be the major vector of *Wuchereria bancrofti* (the nocturnally-periodic strain) in the northern half of the United States in the event that the parasite were to become established there. Its high infectibility rate, domestic breeding habits, and its willingness to feed on man make this species ideally suited as a vector.

A high infectibility rate was obtained with *Psorophora discolor* and this species readily attacks man. However, because it is essentially a rural breeder, its potentiality as a major vector is lessened. In areas where there is considerable irrigation this species becomes very numerous, but the concentration of population in these areas is usually low. Therefore, it would require a rather high incidence of infection within the flight range of this species in order for the latter to play a dominant rôle in the spread of filariasis.

Because of the low infectibility rates obtained with *Anopheles crucians*, *Aedes thibaulti*, and *Culex salinarius*, it is unlikely that any of these species would be incriminated as a major vector of *Wuchereria bancrofti*. It is conceivable that they might serve as occasional transmitters in an area of high endemicity.

*Anopheles maculipennis freeborni*, *Anopheles quadrimaculatus*, and *Psorophora ciliata* would be incapable of transmitting filariasis because of their complete failure to allow normal development of the larvae.

Although the desired goal of 100 late dissections was not obtained with *Mansonia perturbans*, *Psorophora cyanescens*, and *Aedes canadensis*, the negative results obtained from more than 50 late dissections of each species indicated that none is likely to be a successful intermediate host. Studies on *Culex erraticus* likewise were not completed, but 49 late dissections indicated that this species might prove to be a fairly efficient intermediate host. Although this species usually breeds in rural areas, its feeding habits bring it to houses and barns wherein it rests during the day. In this respect it resembles *Anopheles quadrimaculatus*. The species probably feeds on domestic animals most of the time, but it has the reputation of being rather aggressive toward man in

certain areas. These characteristics together with its relatively high infectibility rate make this species a potential vector of filariasis. However, additional dissections are desirable to substantiate this opinion.

Dissections of *Psorophora howardii* and *P. ferox* were too few to warrant any conclusion. All dissections were negative.

#### SUMMARY AND CONCLUSIONS

Studies to determine possible mosquito vectors of *Wuchereria bancrofti* (nocturnally-periodic strain) in the United States were continued. A total of 1314 dissections was made of mosquitoes from 14 species.

Seventy-eight per cent of *Culex pipiens* examined 9½ days or longer after infection contained infective larvae, and an additional 13 per cent contained larvae in late stages of development. A total infectibility rate of 91 per cent was obtained with this species.

Thirty-three per cent of *Psorophora discolor* in late dissections contained infective larvae, and an additional 34 per cent contained larvae in late stages of development. The infectibility rate of this species was 67 per cent.

Occasional development of the larvae to advanced or infective stages was observed in the following species: *Culex salinarius*, 3 per cent; *Anopheles crucians*, 2 per cent; *Aedes thibaulti*, 3 per cent.

No development beyond the first stage was observed in *Anopheles quadrimaculatus*, *Anopheles maculipennis freeborni*, and *Psorophora ciliata*.

Studies were not completed on *Aedes canadensis*, *Culex erraticus*, *Mansonia perturbans*, and *Psorophora cyanescens*. However, on the basis of 50 or more late dissections of each species, none of these was a good intermediate host with the exception of *Culex erraticus* which permitted development of the larvae to late and infective stages in 14 of 49 specimens.

The few dissections obtained of *Psorophora ferox* and *P. howardii* were negative.

It is concluded that *Culex pipiens* and *Psorophora discolor* are capable of serving as vectors of *Wuchereria bancrofti* should other conditions prevail for the spread of the parasite. Incomplete studies on *Culex erraticus* indicate that this species also might be involved as a transmitter although to a lesser extent. *C. salinarius*, *Anopheles crucians*, and *Aedes thibaulti* might serve as occasional vectors, although their low infectibility rates preclude their playing a major rôle in the spread of the disease. *Anopheles quadrimaculatus*, *A. maculipennis freeborni*, and *Psorophora ciliata* are incapable of transmitting infection. Finally, although studies are not completed, it is apparent that *Aedes canadensis*, *Mansonia perturbans*, and *Psorophora cyanescens* could not serve as vectors of *Wuchereria bancrofti*.

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# STUDIES OF FILARIASIS

## DEVELOPMENT OF *WUCHERERIA BANCROFTI* IN *CULEX* *QUINQUEFASCIATUS* OF OAHU

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The study reported here was carried out with the purpose of determining whether *Wuchereria bancrofti* of Okinawans would develop to the infective stage in the mosquito, *Culex quinquefasciatus*, on Oahu, Territory of Hawaii. Some observations made in an experimental feeding of the same species of mosquito on Koreans infected with *W. malayi* are also reported.

In years past Samoans infected with *Wuchereria bancrofti* have come to Oahu, but no evidence of transmission has appeared. Samoan *W. bancrofti* is known to develop best in and is transmitted by *Aedes scutellaris* var. *pseudoscutellaris* (1). This mosquito is absent from Oahu. Western Pacific *W. bancrofti* of the type carried by Okinawans on the other hand appears to develop best in and is transmitted by *Culex quinquefasciatus* (2). This is the most common mosquito on Oahu.

Preliminary to this study a microfilarial survey was made on Okinawan, Korean and Japanese prisoners of war brought to Oahu, T. H. Thick blood films were taken at night. Of 3768 Okinawans 708 (18.7%) were found positive, of 570 Koreans 49 (8.5%) and of 659 Japanese 7 (1.0%) were found positive. The Okinawans and Japanese were positive for *W. bancrofti* and the Koreans for *W. malayi*.

The periodicity was determined by counts of the number of microfilariae in 20 cmm. blood taken at 10 A.M., 2 P.M., 6 P.M. and 10 P.M. Five Okinawans were checked, and a strong nocturnal periodicity of the *W. bancrofti* microfilariae found. The counts of microfilariae at the above hours on a typical Okinawan ran 0, 2, 14, 139. A similar series on five Koreans revealed a similar, though slightly less rigid, periodicity for *W. malayi*. Microfilarial counts on a typical Korean ran 2, 4, 37, 107.

Development in the mosquito of *W. bancrofti* from Okinawans was followed by dissection of laboratory reared mosquitoes after feeding on Okinawans positive for microfilariae. In the course of the day to day dissections photomicrographs were made of the step by step development to provide a series comparable to studies which have been reported from Samoa (1) and the Philippines (2). The photomicrographs are presented in figures 1 to 14. The explanation of these figures follows.

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Figure 1 shows a larva obtained on dissection of a mosquito the day after feeding. The microfilarial sheath is gone and some thickening of the body has



FIG 1



FIG. 2

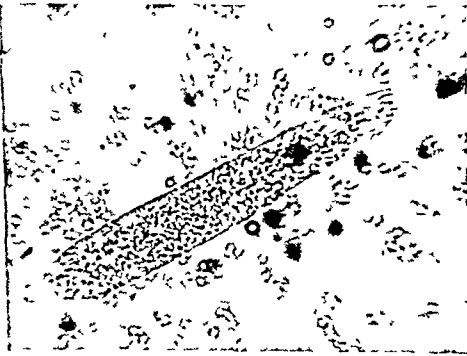


FIG 3



FIG. 4



FIG. 5



FIG. 6

taken place. Initial development is rapid, and by the end of the second day a marked thickening and some internal differentiation may be evident (fig. 2). A prominent tail spike is characteristic of the larva during the period of develop-

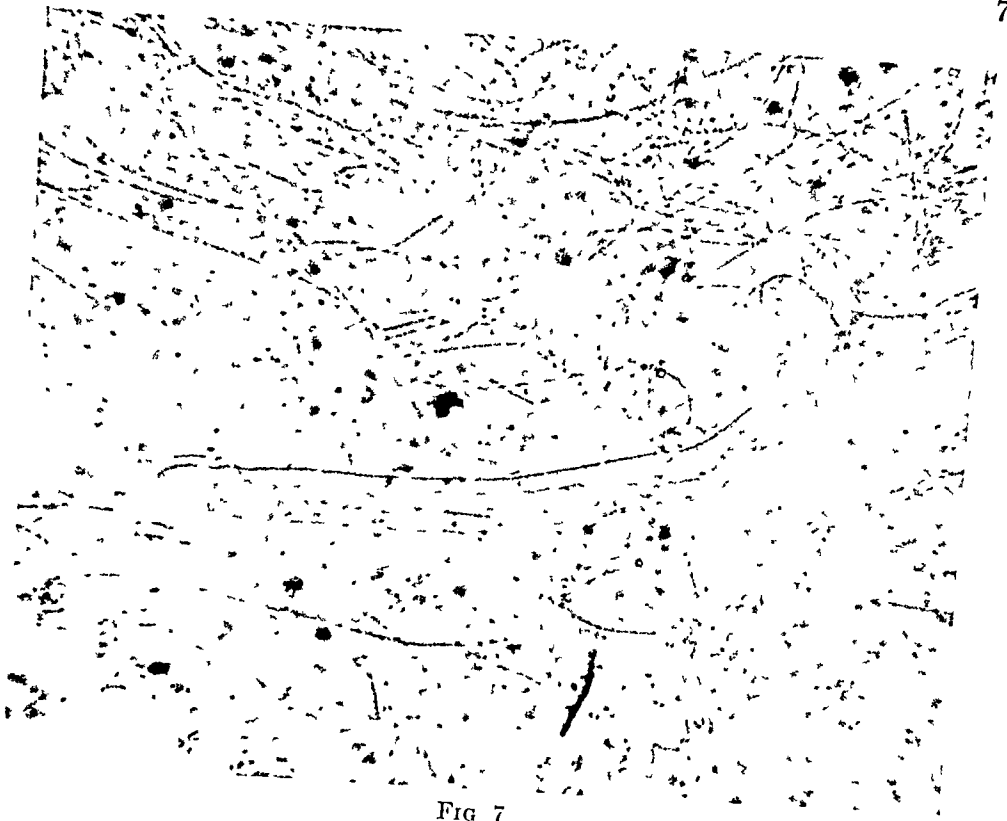


FIG 7

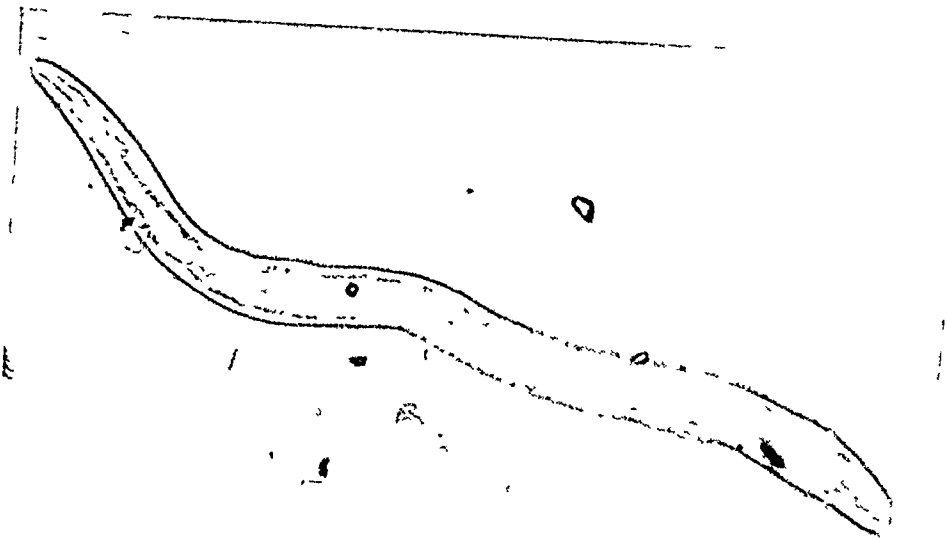


FIG 8

ment through the third day (fig. 3), fifth day (fig. 4) and sixth day (fig. 5). These stages of development take up to the time of the first moult. In figure 4 the tail spike is bent out of focus. The anal vesicle is visible near the posterior end.



FIG. 9

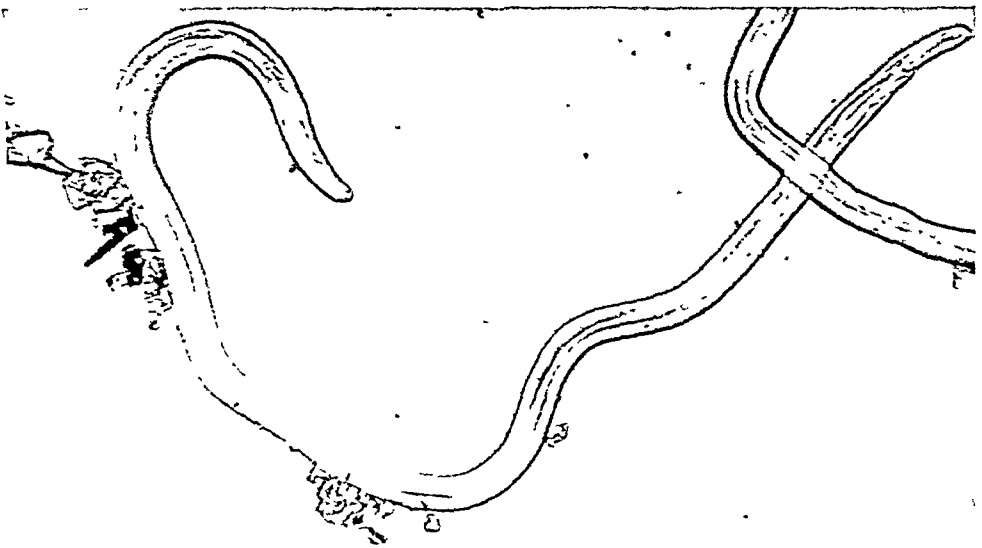


FIG. 10

Figure 6 shows a larva at the end of the seventh day. The first moult has taken place. The spike-like tail projection is lost with the shed cuticula. The digestive tract has become well differentiated, and there is a marked increase in

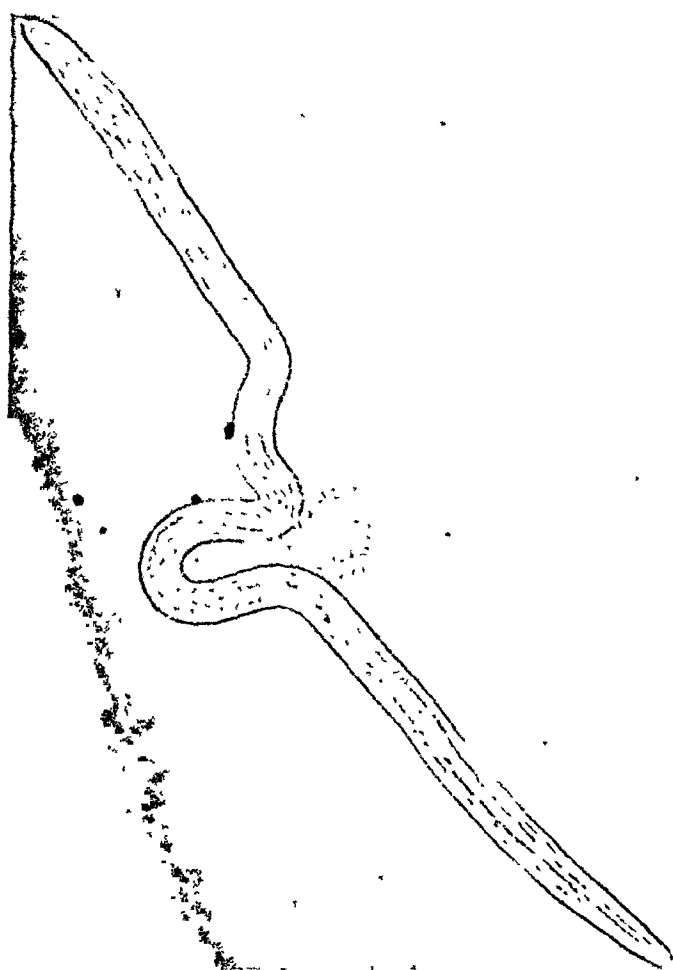


FIG. 11

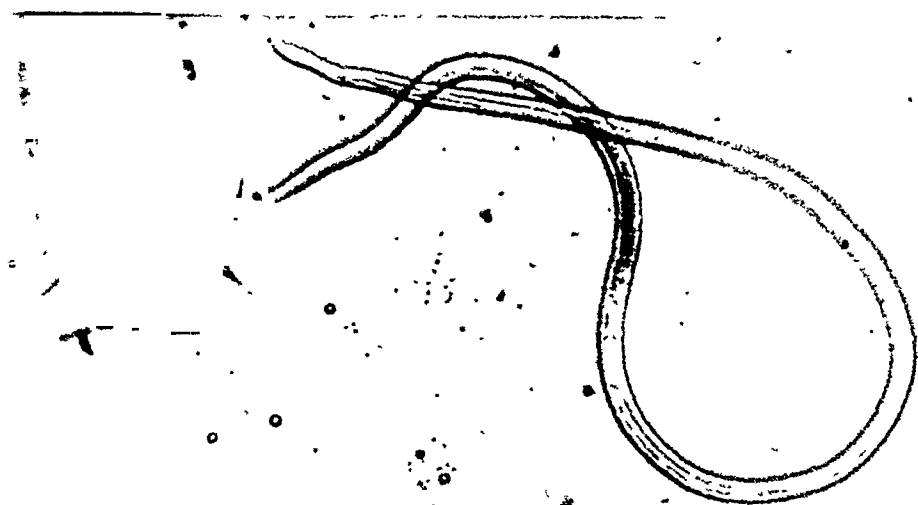


FIG. 12

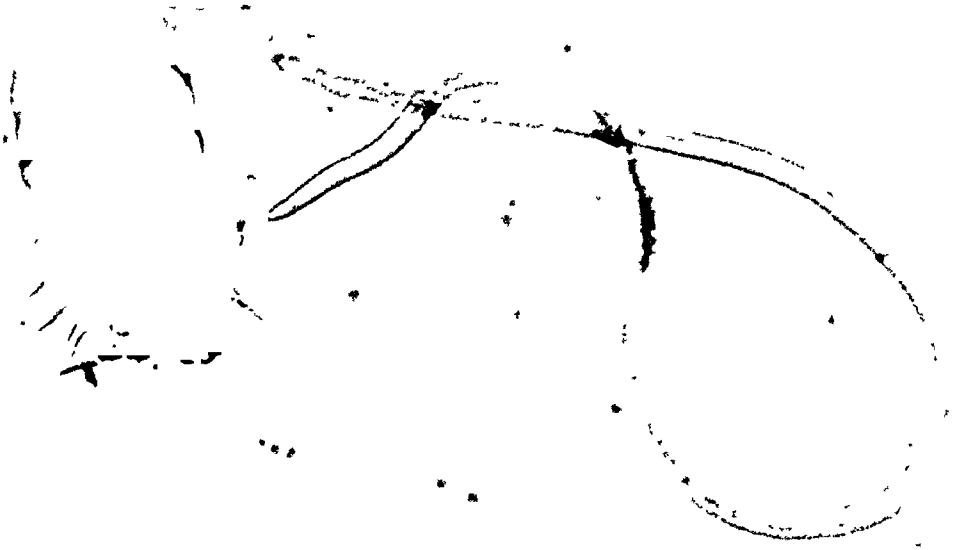


FIG. 13



FIG. 14

length. A small posterior conical projection is apparent during the period of development through the eighth (fig. 7), ninth and tenth days (fig. 8) which takes up to the second moult. Figure 8 shows the tenth day larva considerably compressed to reveal the digestive tract.

Figure 9 shows the larva on the eleventh day after the second moult. Marked growth in length takes place again at this time. Progressive growth and maturing is shown by the stage obtained from the head of a mosquito on the thirteenth day (fig. 10) and fourteenth day (fig. 11). The mature infective larva obtained from the labium of a mosquito on the fifteenth day is illustrated in figure 12 with the anterior end in focus and figure 13 with the posterior end in focus.

Figure 14 shows a mature infective larva projecting from the labium of a mosquito.

Observations on *W. malayi* development in *Culex quinquefasciatus*. *W. malayi* has been shown to undergo its development in six days in certain species of *Mansonia* and *Anopheles* but not in *Culex fatigans* (3). In a feeding trial carried out by us, fourteen laboratory reared *Culex quinquefasciatus* were dissected from the second to the tenth day after feeding on a Korean positive for

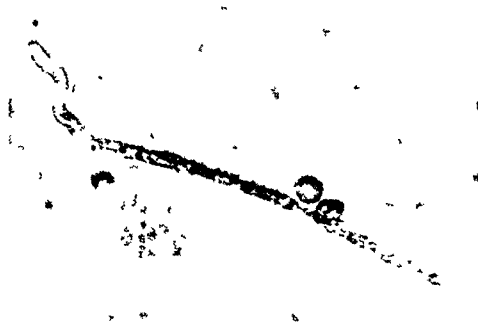


FIG. 15. A PHOTOMICROGRAPH OF A LARVA OF *W. MALAYI* OBTAINED BY DISSECTION OF A *CULEX QUINQUEFASCIATUS* THE DAY AFTER FEEDING ON A POSITIVE KOREAN. This individual is still inclosed in the microfilarial sheath. Note the peculiar, twisted tail.

*W. malayi*. In only one a mosquito dissected on the second day were any larvae found. This is depicted in figure 15.

#### SUMMARY AND CONCLUSIONS

Microfilariae of *Wuchereria bancrofti* from Okinawa develop readily into infective larvae in *Culex quinquefasciatus* of Oahu, Territory of Hawaii. Microfilariae of *Wuchereria malayi* from Koreans failed to develop in the same mosquito.

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# LABORATORY STUDIES ON THE SNAIL HOST OF *SCHISTOSOMA MANSONI*<sup>1</sup>

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In the initial phases of an investigation undertaken in this Laboratory on the susceptibility of American Planorbidae to infection with *Schistosoma mansoni*, Cram, Jones, and Wright (1) in 1944 reported negative results with 11 species and subspecies of planorbids; a total of 100 specimens had been exposed to miracidia without evidence of subsequent infection. At that time attention was called to the fact that *Tropicorbis donbilli*, obtained from Texas, had exerted the greatest attraction for the miracidia; because of this observation and also because of the close relationship of the genera *Tropicorbis* and *Australorbis*, attention had been centered on *T. donbilli*, with unsuccessful attempts to infect a total of 40 specimens.

The following year the same authors (2) reported the first positive results. A species of *Tropicorbis* from Louisiana, identified by U. S. National Museum specialists as *T. havanensis* (Pfeiffer), was established as a laboratory colony. When experimental infection with *S. mansoni* was attempted, in one lot of six artificially reared juveniles, one snail, and in another lot of six, two snails, later shed cercariae. This constituted the first demonstration that we have in the continental United States a species of snail which is capable of serving as an intermediate host of *S. mansoni*.

## ADDITIONAL NEGATIVE RESULTS

Continuing the work, the present writers can now report additional negative results, consistent with the original report (1) for the following number of specimens: *Helisoma duryi intercalare*, 9; *H. subcrenatum* (typical), 8; *H. subcrenatum disjectum*, 7; *H. tenue californiense*, 11; *H. trivolvis*, 38; *Planorbis corneus*, 4; *Tropicorbis donbilli*, 36. Species which had not been previously tested and which also failed to show evidence of infection after exposure include the following: *Gyraulus parvus*, 6; *G. similaris*, 19; *G. stearnsi*, 10; *Helisoma duryi eudiscus*, 19; *H. duryi seminole*, 15; *H. scalare*, 6; *H. tenue sinuosum*, 17; *H. trivolvis lentum*, 16; and *Planorbula armigera*, 14.<sup>2</sup>

<sup>1</sup> Presented at the 20th Annual Meeting of the American Society of Parasitologists, St. Louis, Mo., March 30, 1946.

<sup>2</sup> We are indebted to Dr. Paul Bartsch, Curator of Molluscs and Cenezoic Invertebrates, U. S. National Museum, and to Dr. J. P. E. Morrison of his staff for identification of the species listed here, and to many persons for their cooperation in collecting and shipping snails, the following persons having furnished the species dealt with in this report: G. H. Ball, M. Berg, E. G. Berry, H. S. Colton, S. Dinkins, W. O. Gregg, J. B. Lackey, E. L. Lazier, F. Lyman, P. H. Reed, E. N. Wilcox, F. H. Wilson, and W. H. Wright.



#### FURTHER INVESTIGATION OF THE SUSCEPTIBILITY OF *TROPICORBIS* FROM LOUISIANA

Up to the present time, for these studies we have had four lots of *Tropicorbis* from Louisiana; these were designated by us as Louisiana C, La. K, La. N, and La. O, in our alphabetical filing of snails received from that state.

La. C: This was the lot which was utilized in the initial tests (2); it was collected by Dr. Arlie Todd in September, 1944, "in the University Lake at the edge of Louisiana State University campus which is considered a suburb of Baton Rouge."

The remaining three lots were collected in September, 1945 by Dr. Elmer G. Berry from the following localities:

La. K: Collected from the same lake from which the collection had been made by Dr. Todd the previous year; they were found by Dr. Berry at the extreme south end of the lake, just below the overflow, either floating or on aquatic vegetation close to the surface (2" deep) near the bank, one-half mile east of the Agricultural Center Building on University Campus. The water was warm and murky with pH 7.8.

La. N: Collected from Audubon Park, New Orleans. The snails were found on *Ceratophyllum*, close to the bank, in a lagoon situated near the zoo. The water was stagnant and shallow with pH 8.2.

La. O: Collected at Baton Rouge, southwest of the University, in a borrow pit between the old and new levees; water from the Mississippi River had washed over the new levee into the pit. There was no aquatic vegetation; the snails were found on dead leaves and sticks in water with pH 8.4.

#### SPECIFIC IDENTITY OF LOUISIANA *TROPICORBIS*

Concerning the snails which he collected, as noted above, Dr. Berry has furnished us the following statement: Specimens from Lot K have been determined as *Tropicorbis havanensis* (Pfeiffer). Those from Lots N and O, although resembling each other in shell characters, are not alike in their genital structures, and each is specifically distinct from Lot K (*T. havanensis*). Few anatomical studies have been made on members of this Genus and until further research work has been made the specific identifications of Lots N and O can not be determined.

#### MATERIALS AND METHODS

In order to provide a variety of conditions which might influence the susceptibility of *Tropicorbis* to infection, both wild and laboratory reared snails of various ages were used; some were exposed individually, usually to 3 miracidia and others *en masse* to varying numbers. For some, miracidia of *S. mansoni* originating from rodents and for others those from monkeys were employed. In some instances there was only one exposure and in other cases so-called "prolonged" exposure, miracidia being added to the water with the snails from 2 to 5 times within a 2 to 12 days' period; in addition some snails were reexposed, that is, again exposed to infection at a later date after completion of the incubation period and an observation period with absence of evidence of infection. As

as a rule the snails were held in a 25°C. incubator at night and were taken out into the light at room temperature during the day. Snails which died during the incubation period were dissected in search of sporocysts, if dissolution of the snail body had not taken place. After an incubation period of approximately 22 days, microscopical examinations were made daily for the emergence of cercariae.

#### RESULTS

The number of snails exposed and the results obtained are shown in table 1. In the initial phases of the investigation with snails of La. C lot, laboratory-reared specimens were used with a single exception. The one wild specimen and 17 laboratory-reared specimens were exposed individually and failed to show evidence of infection. On the other hand, of a total of 45 laboratory-reared specimens which comprised 9 groups of 2 to 7 snails each, in which the snails were exposed not individually but as a group, 38 snails gave no evidence of infection whereas 7 specimens shed cercariae of *S. mansoni*. Five of the 38 resistant snails were reexposed at a later date but again with negative results.

The susceptibility of wild snails as compared with those reared in the laboratory was more thoroughly tested with the three lots of *Tropicorbis* received in the autumn of 1945. As noted in table 1, of 21 wild specimens of lot La. K, exposed individually, 20 failed to become infected and 1 subsequently shed cercariae. Of the 20 resistant snails, 7 were reexposed individually with negative results, and of these 2 were exposed a third time and still showed no evidence of infection. However, the remaining 13 of the original 20 negative snails were utilized for reexposure in 3 groups of 4, 4 and 5 snails, respectively, and in one group 1 snail later shed cercariae. Eleven of the 12 resistant snails were exposed a third time, as a group, but again failed to become infected. On the other hand, there were no instances of infection among 15 laboratory-reared snails which were exposed individually, even when 5 of these were exposed a second time.

With snails of the two lots, La. N and La. O, no positive results have been obtained to date. As noted in table 1, principally wild specimens have been employed to the present time. Of La. N snails, 19 were submitted to individual exposure and 24 to group exposures, with later reexposure of 15 and 19 specimens, respectively, from the two categories. Only 5 laboratory-reared specimens have been available; these also failed to shed cercariae after individual exposure. Of wild snails of the La. O lot, 60 were exposed individually with 52 of these later being reexposed and 22 of the latter exposed a third time with consistently negative results; similarly, attempts to infect several groups, totaling 235 specimens, met with failure; 106 of these were exposed a second time without resultant infection. To date, only 12 laboratory-reared specimens have been utilized; these were exposed individually with negative results, even when 6 were subsequently reexposed.

Summarizing the experimental work to date, of a total of 464 Louisiana *Tropicorbis* exposed, 9 snails, or 1.9 percent, subsequently shed cercariae of *S. mansoni*. Of 360 wild specimens, 2 gave positive results, one of these being a specimen which was resistant when first exposed individually but which developed cercariae

after a second exposure when it was one of a group which was exposed *en masse*. On the other hand, of a total of 101 laboratory-reared snails, 7 were successfully infected.

It will be noted from table 1 that all 9 specimens of *Tropicorbis* which were experimentally infected with *S. mansoni* were of the same genetic background,

TABLE 1  
Results of experimental exposure of *Tropicorbis* to *Schistosoma mansoni*

LOT NUMBER AND ORIGIN OF SNAILS	INDIVIDUAL EXPOSURE					GROUP EXPOSURE				
	Wild			Laboratory-reared		Wild			Laboratory-reared	
	Exposed			Exposed		Exposed			Exposed	
	1X	2X	3X	1X	2X	1X	2X	3X	1X	2X
La. C; L.S.U. campus, Baton Rouge	1-			17-					38-	5-
									7+	
La. K; L.S.U. campus, Baton Rouge	20-	7-	2-	15-	5-		12-	11-		
	1+						1+			
La. N; Audubon Park, New Orleans	19-	15-		5-		21-	19-			
La. O; Levee, Baton Rouge	60-	52-	22-	12-	6-	235-	106-			
Total										
4 Lots	100-	74-	24-	59-	11-	259-	137-	11-	38-	5-
3 Localities	1+						1+		7+	

Wild stock. .... 360 exposed, 358 negative, 2 positive (0.6%)

Lab-reared. .... 101 exposed, 97 negative, 7 positive (6.7%)

Total. .... 461 exposed, 455 negative, 9 positive (1.9%)

L.S.U. strain (La. C and La. K):

Wild stock. .... 22 exposed, 20 negative, 2 positive (9%)

Lab-reared. .... 77 exposed, 70 negative, 7 positive (9%)

Total. .... 99 exposed, 90 negative, 9 positive (9%)

\* These 12 snails were part of the 20 previously exposed individually, with negative results.

belonging to the two lots of snails collected from the lake on the University campus at Baton Rouge. Of wild snails collected at that site, 2 of 22, or approximately 9 percent, and of the laboratory-reared progeny of that stock 7 of 77, also approximately 9 percent, proved susceptible to infection with *S. mansoni*. For the most part the individual experiments were conducted with small numbers of snails. The nine snails successfully infected were distributed in five different

Six mice were infected with cercariae of *S. mansoni* from *Tropicorbis*, three of the mice from the laboratory-reared La. C snails and the other three from the wild specimens of La. K. A maximum of 175 adult *S. mansoni* per mouse, were collected. Lesions were similar to those which result from infection with cercariae from the well-established snail host, *Australorbis glabratus*; tremendous numbers of ova were found in the liver and the wall of the intestine.

The adaptability of *S. mansoni* to these two species of planorbids was evident from its passage through the following steps: From *Australorbis* to a rodent to *Tropicorbis* to a rodent to *Australorbis*. On the other hand, that the infection could be maintained by *Tropicorbis* was indicated by successive transfer of the infection from *Tropicorbis* to a rodent to *Tropicorbis* to a rodent.

#### SUMMARY

Nine additional species and subspecies of native American planorbids have been exposed to infection with *Schistosoma mansoni*, with negative results; additional attempts have been made to infect certain species which had been tested earlier.

Additional laboratory studies have been conducted with a species of *Tropicorbis* which has proved susceptible to infection. To date, 9 specimens have been infected; all of these snails came, or were progeny of snails which came, from a lake on the campus of Louisiana State University at Baton Rouge, Louisiana. Subsequent to exposure of 22 wild adult specimens and of 77 laboratory-reared juveniles, 2 and 7 snails, respectively, or 9 percent, shed cercariae of *S. mansoni*. On the other hand, there are described unsuccessful attempts to infect tropicorbids which, however, appear to be different species and which were collected from two different sites.

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# TOLERANCE OF FOWLS FOR MODERATE INFECTIONS OF INTESTINAL HELMINTHS<sup>1</sup>

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It has been known for over a decade that heavy infections of ascarids are harmful to young animals and in some instances may cause their death. Older animals are more resistant and frequently can harbor rather large numbers of the parasites without noticeable harm. Knowledge of tapeworms as etiological factors of disease in young animals is not so well established. Whereas, heavy infections of adult tapeworms have shown definite effects upon the young hosts in some experiments, in others fairly large feedings of larval tapeworms caused little or no disturbance in the growing hosts.

Examinations of fowls in the vicinity of Manhattan, Kansas, in recent years have revealed moderate infections of ascarids and tapeworms in both ailing and apparently normal young fowls. It was to investigate the importance of moderate infections of roundworms and tapeworms in growing chickens that the present studies were made.

## EFFECTS OF ASCARIDIA GALLI ON CHICKENS

Evidence of harmful effects of large infections of the fowl nematode, *Ascaridia galli*,<sup>2</sup> on young chickens was obtained by Ackert and Herrick (1928) who conducted an extensive series of carefully controlled experiments. Large feedings of *A. galli* eggs (500 to 2000) were given to young chickens kept on what was then considered to be an adequate ration. Chickens four to 11 weeks of age became heavily parasitized and showed significant effects of the parasitism. The growth of the fowls was markedly retarded and the mortality rate increased. Previous tests by Ackert and Titus (1924) showed that heavy infections of *A. galli* lowered the blood sugar levels in the young fowls; and in similar tests it was found that the thymus glands were shrunk in young chickens heavily parasitized with these nematodes (Ackert, 1924). From heavy infections of chickens with *Ascaridia* larvae Clapham (1937) noted intestinal lesions, severe enteritis and petechial hemorrhages on the mucous membranes.

These and other harmful effects noted were due to heavy infections of ascaridia larvae resulting from feeding large numbers of embryonated eggs of the nematode.

## METHODS

Day-old White Leghorn chicks were placed in brooders in a tightly screened room used for testing their tolerance to moderate worm infections. An adequate ration and water were kept before the chickens at all times during the experi-

<sup>1</sup> Read at the Forty-First Annual Meeting of the American Society of Tropical Medicine, at Cincinnati, Ohio, November 13-15, 1945.

<sup>2</sup> *Ascaridia galli* is doubtless the same species as *A. lineata*.

ments, except for short periods before weighing and taking blood samples, when the feed was removed.

At the age of 23 days they were weighed and divided into two groups of 15 chicks each matched closely according to weight, one group to be parasitized and the other to be kept as controls. On the following day approximately 200 embryonated eggs of *A. galli* were fed to each chick of that group. Two weeks later while the larvae were still partially buried between the intestinal villi (Ackert, 1923), a 0.5 to 1.0 ml. blood sample was taken from a wing vein of each chick in both groups which had been without feed for 12 to 24 hours. The samples were stored in small test tubes containing enough powdered sodium citrate to prevent clotting. All samples were analyzed for blood sugar within three hours after they had been taken. Hemoglobin was estimated with the New Dare Hemoglobinometer. The feric sulfate microtitration method of Miller and Van Slyke (1936) was utilized to determine blood sugar, duplicate samples being analyzed in each case. Weights were recorded on the days preceding the analyses. Four weeks after infection the chicks were subjected again to the same series of tests. At the termination of the experiment all chicks of both groups were killed and examined for worms.

#### EXPERIMENT I

After the fowls were on the experiment for two weeks the average gain made by the control chickens was of 101.9 g., as compared with a 99 g. average gain in weight of the parasitized group—a negligible difference of 2.9 g. The average blood sugar level of the control fowls at that time was 173.1 mg. per 100 ml. of blood as compared with an average of 170.7 mg. per 100 ml. of blood in the parasitized birds—again a negligible difference. The hemoglobin estimates for the controls averaged 9.4 g. per 100 ml. of blood while that for the controls was 9.6—a difference of only 0.2 g. which was within the range of experimental error. Thus, at the end of two weeks of parasitism when the ascaridia are in the most damaging position—their anterior ends buried in the intestinal mucosa—there were no constant differences in the growth rate, the blood sugar level, or the hemoglobin content between the control and the parasitized chickens.

The comparative results of the tests at the end of four weeks (table 1) were not very different from those after two weeks. The control fowls gained an average of 299 g. as compared with a 297.6 g. average gain for the parasitized group; and the blood sugar level averaged 168.2 for the controls as compared with 163.2 mg. per 100 ml. of blood for the parasitized fowls—a small difference of 5 mg. per 100 ml. of blood. Considering the wide variability in blood sugar levels in the control and in the parasitized groups, this slight difference between the two groups obviously is not significant.

At this time the experiment was terminated and the fowls of both groups killed and examined for the intestinal nematode, *A. galli*. Not a worm was found in a control chicken, but in the parasitized group, every chicken had ascaridia, the infection range being from one to 46 worms, an average of 17.9 ascaridia per fowl which is somewhat more than the number usually found in range chickens.

While the results failed to show constant differences between the control and the parasitized fowls, it seemed possible that there might be individuals that were definitely affected by the parasitism. Table 1 shows that nine of 15 parasitized chickens gained less than their controls, and eight of 15 had both lower blood sugar levels and hemoglobin content. However, only two parasitized fowls, those with 33 and 20 worms respectively, were lower in all three comparisons. Four other chickens with larger infections failed to give indications of constant effects of the parasitism.

TABLE 1

*Data on effects of A. galli on young chickens each parasitized at the age of 24 days with 200 worm eggs*

GAIN AFTER 4 WKS., CONTROLS	GAIN AFTER 4 WKS., PARASITIZED	BLOOD SUGAR AFTER 4 WKS., CONTROLS	BLOOD SUGAR AFTER 4 WKS., PARASITIZED	HEMOGLOBIN AFTER 2 WKS., CONTROLS	HEMOGLOBIN AFTER 2 WKS., PARASITIZED	NUMBER OF WORMS PARASITIZED CHICKENS
grams	grams	mg./100 ml.	mg./100 ml.	gram/100 ml.	gram/100 ml.	
262	284	137	141	9.6	13.7	5
344	280	164	171	8.3	14.2	1
362	338	189	152	8.7	7.6	33
314	273	204	175	10.6	8.8	20
273	325	132	129	10.0	7.4	46
278	336	199	148	7.8	12.8	11
238	318	188	180	12.6	6.8	4
289	254	190	184	9.2	9.6	18
290	290	158	168	9.6	8.9	32
197	304	160	192	8.4	7.5	2
314	193	170	129	8.8	10.6	1
339	337	162	186	10.3	8.2	16
335	305	160	160	9.6	8.9	39
322	329	150	174	9.0	9.9	2
328	298	160	159	8.6	8.9	38
Ave. 299.0	297.6	168.2	163.2	9.4	9.6	17.9

The results indicate that under conditions similar to those in this test, chickens can tolerate for one month moderate infections of the fowl nematode, *A. galli*, without being harmed by the worms.

#### EFFECTS OF RAILLIETINA CESTICILLUS ON CHICKENS

Cram, in 1928, announced the discovery that certain ground beetles may serve as the intermediate hosts for the fowl tapeworm, *Raillietina cesticillus*. Prior to this time, studies on the effects of this tapeworm on chickens were made on naturally infected fowls. Many such animals studied by earlier workers possessed mixed infections not only of various species of tapeworms but also of parasites distantly removed from this class. In studies made by observation and examination of naturally infected fowls, Gutberlet (1916) reported great hunger and thirst, nervousness, emaciated appearance, drooping wings, catarrh

of the intestine and, in heavy infections, anemia. These symptoms were attributed to tapeworms in general.

Staflseth (1935), reporting on the pathology of the host's intestine as found in autopsy of birds naturally infected with *R. cesticillus*, described a condition of capillary congestion with an infiltration of lymphocytes and polynuclear cells in the villi on either side of the crypt occupied by the worm. He found that hemorrhages were not common; however, there was some enteritis in heavily infected birds.

Controlled studies by Ackert and Case (1938) on fowls experimentally infected with the tape worm *R. cesticillus* showed less gain in weight and a lower blood sugar level in the parasitized chickens than in the non-parasitized group. Infections of long standing were reported to cause a significant lowering of the hemoglobin.

Taylor (1933) working with 12 week old chickens artificially infected with *Davainea proglottina* was unable to find any noticeable disturbance of health in birds harboring as many as 3000 to 4000 worms; however, Levine (1938) in infections of approximately the same magnitude found a difference of two to six ounces between the parasitized and non-parasitized groups of chicks from the 35th day to the 136th day of the experiment. In experiments with the tapeworm *Hymenolepis carioca*, Luttermoser (1940) found that two week old chicks infected with 1000 cysticercoids gained as much over a six week period as did the non-infected group.

Variations in the results obtained by different workers as to the extent of deleterious effects of tapeworms on domestic fowls led to an inquiry into the reasons for those variations. Ackert and Reid (1937) introduced the factor of age resistance when they reported young chickens more susceptible to *Railictina cesticillus* than older chickens. Recently, the role of the nutritional status of the host has been introduced as a major factor in the manifestation of deleterious effects from parasite infections in general. Harwood and Luttermoser (1938) showed that chickens infected with *R. cesticillus* gained only two-thirds as much weight in a two week period as their uninfected controls when both groups were fed a manganese deficient diet; however, uninfected chicks gained only slightly more than the parasitized ones when both groups were fed a more adequate diet. Later, Luttermoser and Allen (1942) reported that there were significant differences in weight gained by chickens parasitized with *R. cesticillus* and unparasitized chickens when both groups were placed on a low protein diet. Again, when a parasitized and unparasitized group were fed a more adequate diet high in protein, there was no marked difference in weight.

#### METHODS

The methods for testing effects of moderate sized infections of tapeworms on fowls were similar to those reported above for ascaridia, except that it was necessary to grow the cysticercoid or larval stage of the tapeworm recently described by Wisseman (1945). For this phase, gravid tapeworm segments of *Railictina cesticillus* containing numerous eggs were fed to ground beetles (*Cratacan-*



thus, *Amara*) in which infective cysticeroids developed in 17 to 20 days. About 200 of these larval tapeworms were fed to each of 22 chickens 40 days of age. An equal number of chickens of from this same hatch was kept as controls, all chickens being given the same adequate ration.

TABLE 2

*Effects of tapeworms (Raillietina cesticillus) on growing chickens each parasitized at 40 days of age with about 200 larval worms*

GAIN AFTER 8 WKS., CONTROLS	GAIN AFTER 8 WKS., PARASITIZED	BLOOD SUGAR AFTER 8 WKS., CONTROLS	BLOOD SUGAR AFTER 8 WKS., PARASITIZED	HEMOGLOBIN AFTER 8 WKS., CONTROLS	HEMOGLOBIN AFTER 8 WKS., PARASITIZED	NUMBER OF WORMS PARASITIZED CHICKENS
grams	grams	mc./100 ml.	mg./100 ml.	gram/100 ml.	gram/100 ml.	
980	821	117	147	14.7	10.4	25
837	868	170	164	15.6	13.8	42
683	630	134	145	10.9	11.9	76
791	526	132	144	11.1	14.2	25
857	669	140	173	10.5	14.9	150
1,114	671	147	169	10.7	13.8	58
759	852	200	153	11.4	13.6	133
792	959	143	142	10.9	10.8	33
1,016	623	172	164	12.5	11.4	133
783	668	143	151	13.4	10.2	16
1,064	909	131	172	13.7	11.9	34
1,076	692	140	187	14.8	10.4	39
958	709	155	149	11.3	12.2	56
948	810	148	154	14.5	10.5	40
478	699	149	164	14.3	14.4	31
728	873	159	152	12.6	10.7	161
647	928	152	137	11.2	12.5	16
759	529	163	144	13.2	10.5	90
506	1,026	137	129	13.9	13.3	172
818	981	136	157	11.2	14.3	2
666		149		14.6		
892		195		14.6		
Ave. 825.1	772.1	150.5	154.8	12.8	12.3	61.5

## EXPERIMENT II

The control and parasitized chickens were observed closely for eight weeks when individual weights were again made, samples of blood drawn for the blood sugar level, hemoglobin content and differential counts and the chickens killed for collection of the tapeworms. The results, except those of the differential counts, are given in table 2. As a group the controls grew somewhat faster; they made an average gain of 825.1 g. as compared with 772.1 g. for the parasitized group. Although matched evenly at 40 days of age the control group included four fowls that gained more than 1000 g., while the parasitized group contained but one of these. It, however, had 172 tapeworms, a portion of which are shown in figure 1. The variations in individual gains were so large that the difference in gains in the two groups is not significant.



FIG. 1. PHOTOGRAPH OF A PORTION OF A FOWL'S INTESTINE OPENED TO SHOW ATTACHMENTS OF TAPEWORMS (*RAILLIETINA CESTICILLUS*)  
About normal size

The comparative blood sugar levels were 150.5 mg. per 100 ml. for the controls and 154.8 for the parasitized group—a slight difference. The hemoglobin comparison was similar: an average of 12.8 g. per 100 ml. of blood for the controls

and 12.3 for the parasitized group. No constant differences between the groups were found in the differential counts.

The post mortem examinations showed that the controls had no worms, but that all of the 20 fowls in the parasitized group (two were accidentally lost) had tapeworms, the infection range being from two to 172, an average of 61.5 worms per chicken. This would be considered as a moderately heavy infection. If the period of parasitism had continued for several months, or under conditions of less favorable management, it is possible that infections such as these might have caused deleterious effects readily measurable. These chickens were fed at all times a full ration adequate in vitamins, proteins and minerals including traces of manganese recently found to be essential for the protection of fowls against ill effects of tapeworms (Harwood and Luttermoser, 1938).

In looking for possible trends in effects of the cestode parasitism, a further examination of the data in Table 2 shows that 12 of the 20 parasitized fowls gained less in the eight week period than did their control mates; only nine of the 20 had lower blood sugar levels; but 11 of the 20 had lower hemoglobin content. However, in only one case, the fowl with 90 tapeworms, were possible effects of the cestodes indicated in all three comparisons: retarded growth, lowered blood sugar and reduced hemoglobin content.

It is apparent that with a completely adequate ration and excellent care growing chickens can tolerate moderate infections of the tapeworm *R. cesticillus* for eight weeks without harm to the fowls.

#### SUMMARY

1. Experiments involving parasitized and control fowls were made to test the tolerance of growing chickens to moderate infections of ascarids and tapeworms.

2. The criteria for judging the tolerance included growth, blood sugar level, and hemoglobin content of parasitized fowls comparable to those of the control chickens. The criteria for the tapeworm tests included also differential blood counts.

3. Chickens 23 days of age parasitized with 200 infective eggs of the fowl ascarid, *Ascaridia galli*, were able to tolerate infections of from one to 46 worms without manifesting definite harm from the worms in four weeks. The average of 17.9 ascarids per fowl is above the average infection in range fowls.

4. Growing chickens parasitized at 40 days of age with 200 larval tapeworms (*Railletina cesticillus*) were not significantly harmed by infections of from two to 172 tapeworms during a period of eight weeks. The average of 61.5 tapeworms per fowl is more than an average infection found under conditions of general poultry production.

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# EMERGENCY STERILIZATION OF DRINKING WATER WITH HETEROPOLAR CATIONIC ANTISEPTICS

## II. PERSISTENCE OF GERMICIDAL ACTION WITHIN INTESTINAL TRACT AND REMOVAL OF EXCESS DRUG WITH ADSORBING AGENTS<sup>1, 2</sup>

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The antiseptic action of heteropolar cationic chemicals, or synthetic cationic detergents, against cysts of *Endamoeba histolytica* and against other microorganisms, their relatively low acute oral toxicity, and their relative freedom from inactivation by high concentrations of organic nitrogenous materials and alkalinity have suggested their use for the emergency sterilization of drinking water.

Two objections may be raised to this suggestion, however: (1) Antiseptic action of these chemicals might conceivably be reversed within the gastrointestinal tract; and (2) The slight taste and unknown risk of chronic toxicity would make it desirable to inactivate or remove excess chemical after antiseptic action has taken place.

### I. FAILURE OF ANTISEPTIC ACTION TO BE REVERSED WITHIN THE INTESTINAL TRACT

A heavy growth of *Salmonella enteritidis* was prepared on agar slants and divided into three portions which were made up into heavily turbid suspensions in sterile water and in 1:10,000 and 1:20,000 dilutions of Zephiran. These suspensions were fed as sole source of fluid to groups of 5 mice each over a period of 3 days, freshly-prepared suspensions being supplied twice daily.

Subcultures of these solutions at the time of first feeding them to the mice gave all negative results with the 1:10,000 dilution and some negative and some positive results with the 1:20,000 dilution.

All of the control animals receiving contaminated water died with typical symptoms of enteritis. One of the 5 mice receiving the contaminated solution of 1:20,000 Zephiran died on the eleventh day, while all 5 receiving the contaminated 1:10,000 Zephiran solution survived and remained asymptomatic during the 12-day period of observation. There was therefore no evidence of reversal of germicidal action within the gastrointestinal tract of the mouse.

<sup>1</sup> Aided by the Weingarten Fund of the University of Southern California School of Medicine.

<sup>2</sup> Read at the Annual Meeting of the American Society of Tropical Medicine, at Cincinnati, Ohio, November 14, 1945.

## II. INACTIVATION OR REMOVAL OF EXCESS ANTISEPTIC BY ADSORPTION

A number of colloidal clays<sup>3</sup>, charcoals, and proteins were used as adsorbing agents for a variety of heteropolar cationic antiseptics. In all instances it was found that these materials tended to adsorb the cation from solution and to form relatively coarse aggregates. There appeared usually to be an optimum cation/adsorbing agent ratio at which adsorption and aggregation were at a maximum. Many of the clays studied had an obnoxious taste or sensation in the mouth and some produced gelatinous colloidal solutions which rendered them unsuitable for drinking water. The taste of the protein-containing solutions was found undesirable, and it was also thought that these compounds would probably be sufficiently expensive and difficult to handle to preclude their use as adsorbing agents in drinking water. The charcoals tested were relatively poor adsorbing agents in comparison with the more active colloidal clays, and the large quantities required were readily detectable in the mouth. A more finely divided charcoal might prove more effective.

"Panther Creek Bentonite,"<sup>4</sup> however, was a dry powder which went rapidly and completely into colloidal solution and, with agitation, almost instantaneously adsorbed the tested cations completely. This commercial clay was fractionated by differential sedimentation in water in order to obtain a colloidal solution containing particles having an approximate diameter of one micron. Spectrographic analysis indicated that it consisted chiefly of calcium, magnesium, and aluminum, largely as silicates, with a fair amount of iron and faint traces of lead, zinc, sodium, and titanium. This colloidal clay solution had no appreciable taste and was not antiseptic itself.

The paper filtrates of mixtures of Zephiran and this colloidal clay were tested for the Zephiran cation with brom phenol blue and biologically. Mixtures of Zephiran and colloidal clay resulted on agitation in the formation of a flocculent precipitate, maximum precipitation occurring when the two solutions were used in a ratio of 5 parts of clay to 1 part of Zephiran, by weight. With this ratio a flocculum was almost immediately formed which settled rapidly from solution. The supernatant clear fluid was entirely tasteless and appeared to be nontoxic for mice. Its Zephiran concentration was approximately 1:200,000.

Separation of the supernatant solution from its precipitate might, however, be difficult or impossible under such conditions as would require the emergency chemical sterilization of water. Tests of taste and toxicity were therefore carried out upon whole mixtures and upon the precipitate itself.

Whole mixtures of this colloidal clay with Zephiran or other tested cationic heteropolar antiseptics were essentially tasteless, the only taste being a flatness attributable to the slight excess of clay. The flocculum contained in these whole mixtures was so soft that its physical presence could not be appreciably detected in the mouth or throat. It was found impossible to injure mice with whole mixtures administered orally in a single dose of 0.5 cc.

<sup>3</sup> Suggested to us by Doctor Valco of the Onyx Oil and Chemical Company.

<sup>4</sup> Obtained from the American Colloid Company.

In order to test more conclusively the taste and toxicity of Zephiran adsorbed on this colloidal clay, a clay-Zephiran sediment was prepared in which the concentration of Zephiran was 1:74. This highly concentrated material was tasteless. An acute oral toxicity test was run in which 0.5 cc. of the concentrated Zephiran-clay mixture was administered orally to mice. The dosage of Zephiran adsorbed on clay thus administered represented the equivalent, on a weight for weight basis, of about 100 liters of a 1:5,000 solution of Zephiran or its entire sediment when adsorbed on clay, consumed by a 60-kilogram individual in a single oral dose. None of the mice receiving this stupendous dose exhibited any symptom of toxicity.

Numerous trials indicated that the adsorbed cation was devoid of antiseptic action.

It was therefore concluded that Zephiran and other heteropolar cationic antiseptics are adsorbed almost completely on colloidal clays or other adsorbing agents and that, in the case of the clays tested, the cation is not significantly eluted in the mouth, as indicated by lack of taste, or within the intestinal tract, as indicated by lack of acute oral toxicity in mice.

The possibility that colloidal clays would reverse the accomplished action of Zephiran was examined by titrations in which various concentrations of Zephiran were inoculated with *E. coli* and held at 20°C. At the end of 10 minutes a subculture was made and colloidal clay was immediately thereafter added to make a final ratio of 5 parts clay to 1 part Zephiran, by weight. After various periods of time and amounts of agitation, second subcultures were made. The ED<sub>50</sub> concentrations of Zephiran were calculated by the methods of Reed and Muench (1) before and after addition of clay.

It was found that there was a downward shift in titer of approximately 0.1 log following the addition of clay as opposed to before the addition of clay. The same margin of antiseptic certainty was therefore obtained with an initial concentration of 1:8,000 Zephiran followed by clay as with 1:10,000 Zephiran alone; or with an initial concentration of 1:5,000 Zephiran followed by clay as compared with 1:7,000 Zephiran alone. Since the resulting mixture was in any case tasteless and nontoxic, it seemed that it would be possible to use almost any desired starting concentration of Zephiran and therefore to obtain any desired margin of antiseptic certainty.

#### DISCUSSION

Studies previously cited (2) have indicated the effectiveness of heteropolar cationic antiseptics against amebic cysts and bacteria and their relative freedom from inactivation by organic nitrogenous materials and alkalinity. Results indicated above have demonstrated that treated organisms do not appear to "come alive" within the gastrointestinal tract of the mouse and that these compounds may be almost quantitatively inactivated by adsorption on colloidal clays. One colloidal clay studied in some detail gave considerable promise of practical usefulness in that it had a high adsorbing capacity, went freely into solution from the dry state, and was not objectionable to the taste.

Further studies would be required before this method for the emergency sterilization of drinking water could be considered ready for human trial. It would seem likely that other adsorbing agents might well prove superior to those investigated here. On the basis of studies reported elsewhere (3), it seems probable that other antiseptics of this general type might be more advantageous than Zephiran, to which our studies were largely confined because of the ease with which this compound could be chemically estimated and the large supply of it which we had on hand. Further studies should undoubtedly be conducted upon the chronic toxicity of cation-clay sediments and upon pharmaceutical methods for dispensing solid clay and solid cation so that they would be consecutively released. Numerous uses of these chemicals in the food and dairy industries suggest further studies on the reversal of antiseptic action within the intestinal tract.

#### CONCLUSIONS

1. Heteropolar cationic antiseptics which are effective against gram-negative bacteria and amebic cysts may be removed from solution in a biologically inert state by adsorption on colloidal clays.
2. This adsorption does not significantly reverse the accomplished antiseptic action.
3. Reversal of germicidal action does not appear to occur within the gastrointestinal tract of the mouse.

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# THE CAUSES OF DEATH ON THE ISTHMUS OF PANAMA\*

BASED ON 14,304 AUTOPSIES PERFORMED AT THE BOARD OF HEALTH  
LABORATORY GORGAS HOSPITAL, ANCON, CANAL ZONE, DURING  
THE FORTY YEAR PERIOD 1904-1944

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Causes of death determined by autopsy are among the most reliable of vital statistics if the autopsies represent an adequate sample of deaths in the general population and if they are done properly. Accurate data as to the causes of death in tropical lands are rarely available because of the scarcity of laboratory facilities and because of the difficulty of obtaining permission for autopsy from the natives. Isolated reports of individual diseases tend to emphasize the spectacular rather than the more frequent and more important causes of death. It seemed worthwhile, therefore, to utilize the unique advantages of this institution and compile these data from the records. A brief report based on 4,806 autopsies during the period 1904-1916 was published locally by Clark (1).

During the forty year period 1904-1944, 21,000 bodies were received at the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone, 68 per cent of which were autopsied. (The high autopsy percentage can be explained, in part, by the fact that the undertakers, who are members of the personnel of the Board of Health Laboratory, obtain permission for autopsies.) The bodies came from Gorgas Hospital (known until 1927 as Ancon Hospital), Corozal Hospital, Colon Hospital, Palo Seco Leper Colony, various Army posts, Canal Zone dispensaries, and many were coroner's cases; they represented an adequate proportion of all deaths on the Canal Zone. The autopsies were complete, including examination of the brain, and histologic sections were prepared. The work was done by the dozen pathologists who have been on duty at the laboratory under the aegis of the late Dr. Samuel T. Darling, who was Chief of Laboratory from 1906 to 1915, and Dr. Lewis B. Bates (now Colonel, Medical Corps, Army of the United States), who has been Chief since 1919. Hence there has been a considerable degree of consistency in the method of classifying the causes of death.

Although these autopsy records provide accurate data as to the causes of death on the Canal Zone, when the figures are compared with statistics from other lands due consideration must be given to the peculiar population groups living on the Isthmus. During the construction period of the Panama Canal thousands of young, healthy Negro laborers were "imported" from the West Indies, mostly from the islands of Jamaica and Barbados. After the Canal was completed many of these men were employed on a permanent basis. Their

\*The period covered fell somewhat short of 40 years, the exact dates being October 21, 1904 to November 7, 1943.

<sup>1</sup>Major, M.C., A.U.S. Pathologist.

families followed them to the Isthmus or they married locally. These Negroes constituted 63.3 per cent of the autopsy series. The native Panamanian, for the most part, is a Latin-American mestizo, a mixture of Indian and Spanish blood; this group constituted 10.4 per cent of the autopsy series. The United States group, contributing 12.7 per cent of the autopsy series, was composed of white employees of the Panama Canal who had passed a physical examination, and their families, and also military personnel stationed on the Isthmus. (A large proportion of white patients who suffer from chronic illnesses return to the United States for further treatment or "to die at home.") All other individuals in this series, 13.6 per cent, were classified in a miscellaneous group which included Spaniards and Italians who were employed in the early years of canal construction, Chinese, Hindus, and many others.

The foregoing description will serve as an explanation for the preponderance of males in the autopsy series. The factor of age must not be disregarded when the data are reviewed. It is obvious from the description of the population groups that the patients in this series were younger than in most autopsy series. However, even correlation of age with causes of death of the various race groups would not permit direct comparison of these data with figures from other areas because of the many other local factors.

#### METHOD

At the end of each autopsy protocol the pathologist who performed the autopsy recorded the cause of death and a number based upon that edition of the International List of Causes of Death in current use. In this study a new number was assigned to the recorded cause of death using as a classification the most recent edition of The Manual of the International list of Causes of Death (Fifth Revision, 1938) (2). In order to demonstrate certain trends in causes of death, the autopsies were divided into three equal groups. Group I (1st Third) covers the period 1904-1917 and includes the construction period of the Panama Canal. Group II (2nd Third) covers the period 1917-1931, and Group III (Final Third) from 1931 to 1944.

In reviewing the data certain principles of this classification must be borne in mind:

(1) In listing the final cause of death it was the policy of the department to employ the fundamental pathological cause of death rather than the terminal physiological state. For example, if the patient had diabetic gangrene of an extremity and died in acidosis, the cause of death would be listed as diabetes mellitus, International List No. 61.

(2) Contributory causes do not appear in these data. For example, if the patient had severe hypertensive cardiovascular-renal disease with a terminal cerebral hemorrhage, cardiac decompensation, or uremia, the cause of death would be recorded as hypertensive cardiovascular-renal disease, International List No. 131a.

(3) Although concepts of some diseases have changed considerably in the past forty years, the individual protocols were not reviewed with the purpose

of reassigning different causes of death. The only assignment was the most modern International List number. This was done because four distinct editions of the International List with different numbers had been used during the period surveyed. For example, International List No. 131b, "other chronic nephritis," includes 490 cases in Groups I and II and only 57 in Group III. If the 490 cases had been autopsied more recently, more specific causes of death such as pyelonephritis, renal arteriosclerosis, or glomerulonephritis might have been assigned.

(4) The International List number has been omitted for those diseases which did not occur in this series. These include, among others, cholera, brucellosis, anthrax, tularemia, relapsing fever, Weil's disease, the typhus diseases, kala-azar, and trichinosis.

(5) Diseases may be concealed in unexpected categories. For example, under granulocytosis, International List No. 76a, in table 1 are recorded 7 cases (those cases in which the cause of the agranulocytosis was not established). Yet deaths in which agranulocytosis followed arsenical therapy are recorded under International List No. 179.

(6) Certain causes of death represented "imported" cases. For example, the three patients who died of plague were seamen who acquired the disease elsewhere; two of the three patients who died of rabies were transients.

(7) Percentages have been omitted in order to keep the tables as simple as possible - and because of indecision as to whether to include stillbirth autopsies and deaths during infancy in the calculations. Percentages can be calculated with ease for any disease in which the reader may be interested.

(8) Table 1 can best be understood by those who have had considerable experience with the Manual of the International List of Causes of Death or by those who consult the volume while reviewing the tables. Since no perfect classification of causes of death is available, no apology for employing this system is necessary.

No attempt will be made to discuss the individual diseases, for many of these have been dealt with in published articles and others are being analyzed at the present time (3).

*Acknowledgments:* The assistance of Anita Larson in the collection and tabulation of the data was invaluable. Col. Lewis B. Bates provided facilities and helpful criticism.

TABLE 1  
Summary of causes of death

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
1	Typhoid fever	202	166	24	12	177	25	165	5	8	24
2	Paratyphoid fever	3	1	2	0	1	2	3	0	0	0
3	Plague	3	3	0	0	3	0	1	0	0	2
6	Cerebrospinal (menin- gococcus) meningitis	60	13	37	10	46	14	27	3	20	10
8	Scarlet fever	4	0	2	2	1	3	3	0	1	0
9	Whooping cough	11	1	5	5	3	8	6	4	0	1
10	Diphtheria	25	8	10	7	16	9	18	5	2	0
11	Erysipelas	5	3	2	0	4	1	1	2	1	1
12	Tetanus	23	16	6	1	20	3	19	0	0	4
13b.	Tuberculosis of respira- tory system	1,059	315	428	316	756	303	730	159	30	140
14	Tuberculosis of me- ninges and central nervous system	67	22	25	20	47	20	47	8	5	7
15	Tuberculosis of intes- tines and peritoneum	34	19	10	5	30	4	26	4	2	2
16	Tuberculosis of verte- bral column	14	4	8	2	13	1	13	0	0	1
17a	Tuberculosis of bones	5	5	0	0	5	0	4	1	0	0
17b	Tuberculosis of joints	3	0	2	1	1	2	2	1	0	0
18	Tuberculosis of skin and subcutaneous tissue	1	0	1	0	1	0	0	1	0	0
19	Tuberculosis of lym- phatic system	2	1	1	0	0	2	1	1	0	0
20	Tuberculosis of genito- urinary system	5	3	2	0	5	0	2	1	0	2
21a	Tuberculosis of adrenal glands	2	0	1	1	2	0	1	0	0	1
21b	Tuberculosis of other organs	3	2	0	1	3	0	2	1	0	0
22a	Acute miliary tubercu- losis	243	161	66	16	190	53	199	24	3	17
22b	Other generalized tu- berculosis	293	216	68	9	232	61	252	19	2	20
23	Leprosy	97	2	37	58	72	25	47	38	0	12
24a	Septicemia	131	90	32	9	105	26	96	11	5	19
24b	Pyemia	85	67	16	2	71	14	63	5	4	13
24c	Gas bacillus gangrene	1	1	0	0	1	0	1	0	0	0
25	Gonococcus infection	14	8	3	3	10	4	13	0	0	1
27a	Bacillary dysentery	53	6	33	14	37	16	37	7	6	3
27b	Amebic dysentery	167	132	22	13	149	18	120	15	5	27
27c	Other and unspecified forms of dysentery	93	88	3	2	73	20	76	6	0	11
28a	Benign tertian malaria	7	2	2	3	4	3	4	1	1	1
28b	Quartan malaria	1	0	0	1	0	1	0	0	1	0

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1 ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
28c	Estivo-autumnal ma- laria	467	322	74	71	370	97	215	45	55	152
28d	Malaria (unqualified form)	17	14	3	0	14	3	13	1	1	2
29	Chagas' disease	3	0	0	3	2	1	1	2	0	0
30a	Locomotor ataxia	2	0	2	0	1	1	1	0	1	0
30b	General paralysis of the insane	227	11	138	78	197	30	164	26	2	35
30c	Other syphilis of cen- tral nervous system	55	7	41	7	47	8	35	6	1	13
30d	Aneurysm of aorta (syphilitic)	237	47	89	101	202	35	200	12	6	17
30e	Other syphilis of circu- latory system	31	0	14	17	29	2	24	1	2	4
30f	Congenital syphilis	37	6	22	9	19	18	30	6	0	1
30g	Other and unspecified forms of syphilis	118	30	68	20	91	27	98	12	1	7
32b	Vincent's angina	1	0	1	0	1	0	1	0	0	0
33a	Influenza with respira- tory complications specified	31	0	30	1	20	11	13	12	2	4
33b	Influenza without re- spiratory complica- tions specified	25	2	15	8	14	11	17	7	0	1
34	Smallpox	4	1	3	0	1	3	3	1	0	0
35	Measles	11	3	5	3	8	3	5	5	0	1
36	Acute poliomyelitis and polioencephalitis	9	0	5	4	7	2	0	2	7	0
37a	Acute infectious en- cephalitis (lethargic)	2	0	1	1	2	0	1	0	1	0
37c	Encephalitis lethargica (unqualified)	1	0	1	0	1	0	0	0	0	1
38a	Yellow fever	23	21	2	0	22	1	0	0	3	20
38b	Rabies	3	1	0	2	3	0	0	1	1	1
38c	Herpes zoster	2	0	0	2	2	0	0	0	2	0
38e	Chicken pox	2	0	2	0	2	0	2	0	0	0
40	Ankylostomiasis	22	18	3	1	20	2	8	5	0	9
41	Hydatid disease	3	3	0	0	3	0	0	0	0	3
42	Ascariasis	7	0	4	3	4	3	1	5	0	1
42	Cysticercosis	2	1	1	0	1	1	2	0	0	0
42	Schistosomiasis	1	1	0	0	1	0	1	0	0	0
42	Strongyloidosis	1	0	0	1	1	0	1	0	0	0
43	Actinomycosis	1	0	0	1	1	0	1	0	0	0
43	Histoplasmosis	3	3	0	0	3	0	2	0	0	0
43	Thrush	2	2	0	0	2	0	2	0	0	0
43	Other mycoses	1	0	0	1	1	0	1	0	0	0
44a	Venereal diseases (ex- cept syphilis and gon- orrhea)	5	4	0	1	1	4	5	0	0	0

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	DWI	PAN.	USA	OTH- ERS
44b	Hodgkin's disease	7	1	3	3	7	0	6	0	1	0
44d	Myiasis	2	1	1	0	0	2	1	1	0	0
45b	Cancer of tongue	10	1	4	5	10	0	5	1	1	3
45c	"    " mouth	7	0	1	6	6	1	4	0	3	0
45d	"    " jaw bone	3	0	1	2	2	1	2	0	1	0
45e	"    " unspecified parts of buccal cavity	4	2	1	1	3	1	2	1	0	1
45f	Cancer of pharynx	7	0	3	4	5	2	4	0	2	1
46a	Cancer of esophagus	47	1	16	30	41	6	41	0	4	2
16b	"    " stomach	115	27	35	53	99	16	84	6	10	15
46c	"    " duodenum	2	0	2	0	2	0	1	0	0	1
46d	"    " rectum and anus	12	1	0	11	11	1	2	1	7	2
46e	Cancer of intestines (except duodenum and rectum	15	2	1	12	11	4	7	1	3	4
46f	Cancer of liver and biliary passages	55	5	21	29	42	13	31	5	7	12
46g	Cancer of pancreas	21	0	6	15	16	5	13	0	6	2
43h	"    " mesentery and peritoneum	4	1	3	0	3	1	1	1	2	0
47a	Cancer of larynx	10	0	4	6	10	0	3	2	2	3
47b	"    " trachea	1	1	0	0	1	0	0	0	0	1
47c	"    " bronchus	8	0	2	6	5	3	5	0	3	0
47d	"    " lung	17	0	7	10	13	4	9	0	5	3
47f	"    " mediastinum	3	1	0	2	2	1	1	0	2	0
48a	"    " cervix	36	1	15	20	0	36	27	2	2	5
48b	"    " fundus	19	2	9	8	0	19	16	1	0	2
49a	"    " ovary	4	0	1	3	0	4	1	0	1	2
49c	"    " vagina	2	0	1	1	0	2	1	1	0	0
49e	"    " other sites	6	5	0	1	0	6	4	1	0	1
50	"    " breast	26	2	13	11	1	25	24	1	0	1
51a	"    " scrotum	2	0	0	2	2	0	2	0	0	0
51b	"    " prostate	30	1	6	23	30	0	29	0	0	1
51c	"    " testes	1	0	0	1	1	0	1	0	0	0
51d	"    " penis	3	1	2	0	3	0	2	0	0	1
52a	"    " kidney	3	0	3	0	3	0	1	1	0	1
52b	"    " bladder	22	0	6	16	20	2	16	1	3	2
53	"    " skin	4	0	1	3	3	1	4	0	0	0
54a	Glioma of central nerv- ous system	13	1	4	8	8	5	4	3	6	0
54b	Other cancers of central nervous system	6	2	4	0	3	3	4	1	0	1
55a	Cancer of adrenal	3	0	2	1	3	0	3	0	0	0
55b	"    " bone	6	3	2	1	6	0	6	0	0	0
55c	"    " thyroid gland	1	0	0	1	1	0	0	0	0	1
55d	Cancer of nasal cavity and accessory sinuses	4	1	1	2	4	0	2	0	1	1
55e	Cancer of other organs	64	17	24	23	52	12	45	4	4	11

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
56a	Nonmalignant tumors of ovary	5	2	2	1	0	5	4	1	0	0
56b	Nonmalignant tumors of uterus	10	2	3	5	0	10	9	0	0	1
56d	Nonmalignant tumors of central nervous system	15	1	8	6	11	4	9	1	4	1
56e	Nonmalignant tumors of other organs	6	1	2	3	5	1	4	0	2	0
57d	Unspecified tumors of central nervous sys- tem	3	2	1	0	2	1	1	0	1	1
58b	Acute rheumatic endo- carditis	2	0	0	2	2	0	0	0	2	0
58c	Acute rheumatic myo- carditis	2	0	1	1	1	1	2	0	0	0
58d	Other acute rheumatic heart diseases	8	0	1	7	2	6	6	1	1	0
58e	Other forms of acute rheumatic fever	1	0	0	1	1	0	1	0	0	0
60	Gout	1	0	1	0	0	1	1	0	0	0
61	Diabetes mellitus	68	5	30	33	29	39	57	1	4	6
62	Diseases of pituitary gland	2	1	1	0	1	1	1	0	1	0
62b	Exophthalmic goiter	6	0	1	5	1	5	4	0	1	1
62d	Other diseases of thy- roid glands	1	1	0	0	0	1	0	1	0	0
64	Diseases of thymus	13	3	2	8	6	7	8	3	2	0
62b	Diseases of adrenal glands (except Addi- son's disease)	2	0	0	2	2	0	1	0	1	0
66b	Other general diseases	6	0	3	3	2	4	5	1	0	0
67	Scurvy	2	2	0	0	2	0	2	0	0	0
68	Beriberi	20	12	7	1	18	2	10	2	0	8
69	Pellagra (except alco- holic)	91	34	54	3	17	74	87	0	1	3
70	Rickets	8	1	5	2	6	2	7	0	0	1
72a	Primary purpuras	7	2	3	2	6	1	3	1	2	1
72b	Hemophilia	6	1	4	1	5	1	3	1	2	0
73a	Pernicious anemia	3	1	1	1	2	1	2	0	0	1
73b	Other hyperchromic anemias	1	1	0	0	1	0	1	0	0	0
73c	Hypochromic anemias	3	1	0	2	3	0	2	1	0	0
73d	Other anemias (includ- ing sickle cell anemia)	53	19	19	15	31	22	40	7	1	5
74a	Leukemias	27	5	13	9	22	5	10	3	5	9
74b	Aleukemias	1	0	0	1	1	0	0	0	1	0
75a	Splenic anemia	3	0	2	1	3	0	0	2	1	0

TABLE 1—*Continued*

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
75b	Splenomegaly (of unde- termined nature)	5	0	3	2	5	0	1	2	1	1
75c	Other diseases of spleen	6	4	1	1	5	1	4	0	1	1
76a	Agranulocytosis	7	0	3	4	6	1	0	1	5	1
77b	Deficiency states associ- ated with alcoholism (except pellagra)	11	9	0	2	7	4	8	0	1	2
77c	Acute alcoholism	23	6	5	12	21	2	4	2	11	6
77d	Chronic alcoholism	13	3	4	6	12	1	5	0	8	0
77e	Alcoholism, type (un- specified)	13	4	3	6	12	1	2	1	6	4
78a	Lead poisoning (occu- pational)	2	0	1	1	2	0	2	0	0	0
78b	Lead poisoning (nonoc- cupational)	1	0	1	0	1	0	0	0	0	1
79b	Chronic mercury poi- soning	1	0	0	1	1	0	1	0	0	0
80a	Intracranial abscess	18	4	8	6	15	3	12	0	4	2
89b	Other encephalitis (non- epidemic)	23	9	5	9	14	9	15	1	5	2
81a	Simple meningitis (not due to meningo- coccus)	181	83	54	44	139	42	127	25	9	20
82	Diseases of spinal cord	16	5	6	5	10	6	6	3	3	4
83a	Cerebral hemorrhage	270	45	93	132	190	80	179	16	42	33
83b	Cerebral embolism and thrombosis	29	5	6	18	24	5	22	1	4	2
83c	Cerebral softening	101	28	36	37	67	34	78	8	6	9
83d	Hemiplegia and other paralysis of unspeci- fied origin	5	0	2	3	3	2	4	1	0	0
84a	Mental deficiency	7	1	4	2	6	1	4	3	0	0
84b	Schizophrenia	24	5	12	7	16	8	9	6	1	8
84c	Manic-depressive psy- chosis	8	2	5	1	4	4	5	1	1	1
84d	Other mental diseases	25	18	5	2	13	12	19	3	0	3
85	Epilepsy	23	2	15	6	17	6	9	7	3	4
86	Convulsions (—5 years)	10	2	6	2	5	5	6	1	2	1
87c	Paralysis agitans (ex- cept result of encephalitis)	3	0	1	2	3	0	2	0	1	0
87d	Disseminated sclerosis	4	1	1	2	2	2	3	1	0	0
87e	Other diseases of nerv- ous system	10	3	1	6	6	4	6	3	1	0
88	Diseases of organs of vision	2	0	1	1	1	1	0	1	0	1
89a	Otitis and other dis- eases of ear	51	10	15	26	30	21	36	11	2	2
89b	Diseases of mastoid process	9	1	5	3	5	4	3	1	2	3



TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
90b	Pericarditis (not rheumatic)	34	25	7	2	28	6	24	0	2	8
91a	Bacterial endocarditis (except rheumatic)	71	14	25	32	57	14	43	2	17	9
91b	Other acute or subacute endocarditis (except rheumatic)	20	5	10	5	18	2	15	2	3	0
91c	Other endocarditis, unspecified (—45 years)	2	0	2	0	1	1	2	0	0	0
92a	Diseases of aortic valve (without mention of rheumatic fever)	28	4	17	7	26	2	19	1	2	6
92b	Diseases of mitral valve	10	2	3	5	7	3	6	0	3	1
92c	Diseases of other valves, rheumatic	5	0	2	3	5	0	0	1	3	1
92d	Diseases of other valves (not specified as rheumatic)	27	2	12	13	22	5	16	0	4	7
93a	Acute myocarditis (except rheumatic)	22	2	11	9	15	7	12	6	4	0
93b	Myocarditis (not specified as rheumatic) (—45 years)	10	1	8	1	7	3	7	0	1	2
93c	Chronic myocarditis, rheumatic	2	0	0	2	2	0	1	0	1	0
93d	Chronic myocarditis (not specified as rheumatic)	278	6	48	224	193	85	213	7	38	2
93e	Other myocarditis, unspecified (+45 years)	7	0	4	3	6	1	4	0	1	2
94a	Diseases of coronary arteries	216	6	35	175	186	30	65	6	117	28
94b	Angina pectoris	10	3	6	1	8	2	2	0	6	2
95b	Other diseases of heart, rheumatic	7	0	6	1	5	2	4	1	2	0
95c	Other diseases of heart (not specified as rheumatic)	277	139	73	65	230	47	207	14	15	41
96	Aneurysm	42	3	6	33	35	7	18	2	12	10
97	Arteriosclerosis	108	23	36	49	75	33	73	15	6	14
98	Gangrene	9	3	5	1	8	1	8	0	0	1
99	Other diseases of arteries	10	2	3	5	8	2	4	0	5	1
100a	Varices	2	0	1	1	2	0	1	0	0	1
100b	Other diseases of veins	4	1	2	1	4	0	4	0	0	0
101	Lymphadenitis, nonvenereal	1	1	0	0	1	0	1	0	0	0
102	Hypertension	1	0	1	0	0	1	0	1	0	0
103	Other diseases of circulatory system	4	1	3	0	3	1	4	0	0	0

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	DWI	PAN.	USA	OTH- ERS
104a	Diseases of nasal fossae	3	0	3	0	2	1	2	0	0	1
104b	Diseases of accessory sinuses	26	1	10	15	21	5	15	3	4	4
105	Diseases of larynx	8	2	6	0	7	1	4	1	3	0
106a	Bronchitis, acute	6	2	2	2	4	2	3	2	0	1
106b	“ chronic	9	3	1	5	6	3	4	2	2	1
106c	“ unspecified	2	1	1	0	2	0	1	0	0	1
107	Bronchopneumonia	358	116	125	117	246	112	232	62	24	40
108	Lobar pneumonia	1,011	728	204	79	939	72	736	104	17	154
110a	Empyema	47	16	18	13	38	9	30	6	3	8
110b	Other forms of pleurisy	15	8	2	5	11	4	11	1	1	2
111a	Hemorrhagic infarction and thrombosis of lungs	7	0	1	6	4	3	6	0	1	0
111b	Acute edema of lungs	2	1	0	1	1	1	2	0	0	0
111c	Chronic and unspeci- fied congestion of lungs	8	2	4	2	6	2	4	1	1	2
112	Asthma	8	1	3	4	5	3	3	0	3	2
113	Pulmonary emphysema	3	2	0	1	3	0	1	1	0	1
114c	Gangrene of lung	68	47	16	5	53	15	37	11	2	18
114d	Abscess of lung	27	11	5	11	22	5	18	1	3	5
114e	Other diseases of re- spiratory system	10	1	6	3	8	2	3	2	2	3
115a	Diseases of teeth and gums	3	1	0	2	3	0	3	0	0	0
115b	Septic sore throat	5	1	3	1	3	2	2	0	2	1
115c	Diseases of pharynx and tonsils	15	1	7	7	13	2	6	1	6	2
115d	Diseases of other parts of buccal cavity	16	8	7	1	9	7	12	1	1	2
116	Diseases of esophagus	6	2	3	1	5	1	5	0	1	0
117a	Ulcer of stomach	22	1	9	12	20	2	14	0	8	0
117b	“ “ duodenum	81	22	26	33	74	7	54	1	14	12
118	Other diseases of stom- ach (except cancer)	5	2	2	1	5	0	2	2	0	1
119a	Diarrhea and enteritis (-2 years)	173	76	68	29	88	85	135	18	13	7
119b	Ulceration of intestines (except duodenum) (-2 years)	9	3	2	4	5	4	5	4	0	0
120a	Diarrhea and enteritis (+2 years)	45	25	11	9	32	13	33	9	1	2
120b	Ulceration of intestines except duodenum (+2 years)	20	11	2	7	14	6	15	0	3	2
121	Appendicitis	79	9	39	31	68	11	30	2	27	20
122a	Hernia	32	7	11	14	29	3	19	2	4	7

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
122b	Intestinal obstruction	54	19	17	18	39	15	31	5	9	9
123	Other diseases of intestines	30	8	12	10	16	14	22	2	4	2
124a	Cirrhosis of liver (with mention of alcoholism)	1	0	0	1	1	0	0	0	1	0
124b	Cirrhosis of liver (without mention of alcoholism)	60	13	24	23	51	9	34	2	15	9
125a	Acute yellow atrophy of liver (nonpuerperal)	14	2	4	8	10	4	6	1	2	5
125b	Other diseases of liver	26	10	8	8	21	5	14	1	4	7
126	Biliary calculi	18	2	6	10	11	7	7	2	4	5
127a	Cholecystitis	10	4	2	4	9	1	4	1	1	4
127b	Other diseases of gall-bladder and biliary ducts	1	0	0	1	1	0	0	0	1	0
128	Diseases of pancreas (except diabetes mellitus)	16	2	9	5	13	3	5	0	6	5
129	Peritonitis (cause not stated)	48	20	19	9	30	18	37	4	2	5
130	Acute nephritis	92	47	28	17	75	17	66	8	7	11
131a	Arteriosclerotic kidney	95	9	6	80	79	16	80	2	5	8
131b	Other chronic nephritis	547	282	208	57	433	114	454	35	15	43
132	Nephritis unspecified (10 years +)	7	2	4	1	5	2	4	1	1	1
133a	Pyelitis, pyelonephritis and pyelocystitis	89	36	31	22	59	30	67	9	3	10
133b	Other diseases of kidneys and ureters	55	20	23	12	36	19	35	4	5	11
134a	Calculi of kidneys and ureters	11	2	4	5	10	1	6	0	5	0
134b	Calculi of bladder	4	1	2	1	3	1	2	0	1	1
135a	Cystitis	7	3	2	2	7	0	4	0	2	1
136a	Stricture of urethra	39	15	9	15	39	0	32	1	1	5
136b	Other diseases of urethra	6	3	2	1	6	0	6	0	0	0
137a	Hypertrophy of prostate	21	0	10	11	21	0	17	0	1	3
137b	Other diseases of prostate	11	1	5	5	11	0	6	1	2	2
138	Diseases of other male genital organs (non-venereal)	6	3	1	2	6	0	3	0	1	2

TABLE 1--Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	DWI	PAN.	USA	OTH- ERS
139a	Diseases of ovaries, fal- lopian tubes and parametria	22	6	10	6	0	21	18	0	0	4
139b	Diseases of uterus	4	1	3	0	0	4	4	0	0	0
139c	Diseases of other fe- male genital organs	3	1	2	0	0	3	3	0	0	0
140b	Abortion (—28 weeks) with mention of infec- tion other than pye- litis	4	1	1	2	0	4	3	0	0	1
141a	Abortion (—28 weeks) with hemorrhage, trauma, or shock, and toxemia	1	0	0	1	0	1	1	0	0	0
141b	Abortion (—28 weeks) with hemorrhage, trauma, or shock, but not toxemia	2	0	0	2	0	2	2	0	0	0
141d	Abortion (—28 weeks) without hemorrhage, trauma, shock, or toxemia	2	0	2	0	0	2	2	0	0	0
142b	Ectopic gestation with- out mention of infec- tion	9	3	4	2	0	9	7	2	0	0
143b	Premature separation of placenta (death be- fore delivery)	1	1	0	0	0	1	0	0	0	1
144a	Eclampsia of pregnancy (death before de- livery)	6	3	3	0	0	6	3	3	0	0
144b	Albuminuria and ne- phritis of pregnancy (death before de- livery)	2	0	1	1	0	2	2	0	0	0
144d	Other toxemias of preg- nancy (death before delivery)	16	1	8	7	0	16	13	3	0	0
145	Other diseases and acci- dents of pregnancy (death before de- livery)	3	1	2	0	0	3	3	0	0	0
146a	Placenta praevia (with childbirth)	2	1	0	1	0	2	1	0	0	1
146b	Premature separation of placenta (with childbirth)	2	0	1	1	0	2	1	0	1	0

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
146c	Other hemorrhages of childbirth and the puerperium	6	1	3	2	0	6	5	1	0	0
147b	General or local puerperal infection (except pyelitis)	30	18	9	3	0	30	21	8	0	1
148a	Puerperal eclampsia	19	6	11	2	0	19	10	3	1	5
148b	Puerperal albuminuria and nephritis	4	2	2	0	0	4	4	0	0	0
149a	Trauma of pelvic organs (of childbirth)	7	1	5	1	0	7	2	5	0	0
151	Carbuncle and furuncle	14	3	3	8	12	2	3	2	7	2
152	Phlegmon and acute abscess	34	7	18	9	23	11	22	6	2	4
153	Other diseases of skin and cellular tissue	10	1	7	2	4	6	6	2	1	1
154a	Osteomyelitis and periostitis, acute	5	0	5	0	4	1	3	2	0	0
154b	Osteomyelitis and periostitis, chronic	14	4	7	3	10	4	9	4	0	1
155	Osteopetrosis	1	0	0	1	1	0	1	0	0	0
156a	Diseases of joints (except tuberculosis and rheumatism)	9	5	3	1	7	2	7	0	0	2
156b	Acute myositis	1	1	0	0	1	0	1	0	0	0
157a	Congenital hydrocephalus	13	0	8	5	8	5	8	2	3	0
157b	Spina bifida and meningocele	3	0	0	3	1	2	0	0	3	0
157c	Anencephalus	1	1	0	0	0	1	1	0	0	0
157d	Other congenital malformations of central nervous system	6	1	3	2	3	3	6	0	0	0
157e	Congenital malformations of heart	61	3	27	31	35	26	41	8	6	6
157f	Other congenital malformations of the cardiovascular system	2	0	0	2	2	0	1	0	1	0
157g	Congenital malformations of digestive system	15	1	6	8	11	4	8	1	5	1
157h	Congenital malformations of genito-urinary system	4	0	1	3	4	0	2	1	1	0
157m	Other congenital malformations	29	5	18	6	16	13	20	7	2	0
158	Congenital debility (-1 year)	277	46	200	31	155	122	249	17	7	4

TABLE 1—Continued

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	DWI	PAN.	USA	OTH- ERS
159	Premature birth	214	15	79	120	115	99	141	26	36	11
160a	Intracranial or spinal hemorrhage (—1 month)	52	4	13	35	30	22	28	17	5	2
160b	Other intracranial or spinal injuries at birth (—1 year)	3	0	1	2	2	1	1	0	2	0
160c	Other injuries at birth (—1 year)	18	1	7	10	13	5	13	3	2	0
161a	Atelectasis (—1 year)	34	0	13	21	22	12	25	5	4	0
161b	Infection of umbilicus; pemphigus, sepsis (—1 year)	10	1	9	0	7	3	7	3	0	0
161c	Other specified diseases (—1 year)	54	2	25	27	38	16	41	9	3	1
162a	Senility with dementia	3	0	0	3	1	2	1	1	0	1
162b	Senility without men- tion of dementia	8	4	2	2	5	3	5	2	0	1
163	Suicide by poisoning	35	3	8	24	19	16	5	3	20	7
164a	“ “ hanging	30	2	7	21	28	2	5	3	13	9
164b	“ “ drowning	19	2	8	9	14	5	5	0	9	5
164c	“ “ firearms	120	3	33	84	117	3	2	3	95	20
164d	“ “ cutting in- struments	12	1	6	5	11	1	2	2	3	5
164e	Suicide by jumping	5	0	2	3	5	0	1	0	4	0
164f	“ “ crushing	2	0	0	2	2	0	1	0	1	0
166	Homicide by firearms	40	2	14	24	38	2	7	1	23	9
167	“ “ cutting instruments	25	9	7	9	23	2	6	4	6	9
168	Homicide by other means	35	11	12	12	33	2	10	2	11	12
169	Railway accidents	154	102	13	39	148	6	75	9	24	47
170c	Automobile accidents	227	3	40	184	211	16	64	44	86	33
170d	Motorcycle “	6	1	0	5	6	0	0	0	5	1
171a	Streetcar “	2	0	0	2	2	0	0	0	2	0
171b	Other road-transport accidents	5	0	3	2	4	1	1	0	4	0
172	Water-transport acci- dents	89	2	11	76	85	4	22	15	27	25
173	Air-transport accidents	135	0	13	122	134	1	2	1	129	3
174	Accidents in mines and quarries	10	8	0	2	10	0	5	2	1	2
176	Other accidents involv- ing machinery	28	8	2	18	28	0	15	3	6	4
177	Food poisoning	2	0	0	2	2	0	1	1	0	0
178	Accidental absorption of poisonous gas	2	0	2	0	2	0	2	0	0	0
179	Accidental poisoning by solids or liquids	50	3	26	21	40	10	26	2	17	5

TABLE 1—*Concluded*

INT. LIST NO.	CAUSE OF DEATH	TOTAL CASES	NO. IN 1ST THIRD	NO. IN 2ND THIRD	NO. IN FINAL THIRD	MALES	FE- MALES	BWI	PAN.	USA	OTH- ERS
181	Accidental burns	47	15	17	15	31	16	21	11	9	6
182	Accidental mechanical suffocation	3	0	3	0	2	1	1	1	0	1
183	Accidental drowning	247	17	70	160	238	9	59	31	102	55
184	“ injury by firearms	52	5	13	34	49	3	11	5	32	4
185	Accidental injury by cutting instruments	8	3	1	4	8	0	1	3	3	1
186a	Accidental fall	154	29	48	77	145	9	54	16	51	33
186b	“ crushing	73	35	14	24	71	2	32	10	7	24
188	Injury by animals	10	4	4	2	10	0	4	0	5	1
189	Hunger or thirst	1	0	1	0	1	0	1	0	0	0
191	Excessive heat	7	0	1	6	7	0	1	1	4	1
192	Lightning	7	0	1	6	7	0	3	3	1	0
193	Accidents due to elec- tric currents	21	5	4	12	21	0	4	1	14	2
194	Poisoning by venomous animals (snakes)	5	0	3	2	5	0	1	1	1	2
195a	Sequelae of immuniza- tion inoculation or vaccination	1	0	1	0	1	0	0	1	0	0
195b	Other medical or surgi- cal accidents	13	4	5	4	8	5	11	1	1	0
195c	Neglect of newborn	2	0	1	1	0	2	2	0	0	0
195d	Obstruction, suffoca- tion, or puncture by ingested objects	10	2	4	4	7	3	3	1	2	4
195e	Other accidents	126	57	36	33	120	6	56	8	27	35
198	Legal executions	4	0	4	0	4	0	2	1	0	1
199	Sudden death	1	1	0	0	1	0	1	0	0	0
200a	Ill-defined	125	65	48	12	92	33	84	13	6	22
200b	Found dead (cause un- known)	3	1	0	2	3	0	1	2	0	0
200c	Unknown	136	66	41	29	101	35	78	10	8	40
Total		13,687	4,758	4,451	4,478	10,539	3,148	8,677	1,362	1,747	1,901
	Stillbirth due to acci- dent of pregnancy	88	1	46	41	55	33	43	20	15	10
	Stillbirth due to acci- dent of labor	94	0	47	47	46	48	64	16	8	6
	Stillbirth due to disease of mother	87	2	33	52	48	39	48	23	11	5
	Stillbirth, cause unde- termined	302	10	157	135	165	137	188	60	31	23
	Nonviable fetus	46	0	34	12	26	20	25	8	10	3
Total stillbirths .....		617	13	317	287	340	277	368	127	75	47
Grand total.....		14,304	4,771	4,768	4,765	10,879	3,425	9,045	1,489	1,822	1,948

TABLE 2  
*Most important causes of death*

		TOTAL	1ST THIRD	2ND THIRD	FINAL THIRD
Cardiovascular-renal.....		2,497	885	763	849
Cardiac.....	1,026				
Renal.....	885				
Cerebral accidents.....	405				
Vascular.....	181				
Violent or accidental.....		1,824	336	438	1,050
Tuberculosis.....		1,731	748	612	371
Pneumonia (including empyema).....		1,416	860	347	209
Syphilis.....		707	101	374	232
Cancer.....		635	86	217	332
Diseases of infancy..... (-1 year exclusive of congenital mal- formations and stillbirths)		662	69	347	246
Total.....		9,472	3,085	3,008	3,289

TABLE 3  
*Deaths due to "Tropical" diseases*

	TOTAL	1ST THIRD	2ND THIRD	FINAL THIRD
Malaria (all forms).....	492	338	79	75
Dysentery.....	313	226	58	29
Typhoid and paratyphoid fever.....	205	167	26	12
Leprosy.....	97	2	37	58
Yellow fever.....	23	21	2	0
Ankylostomiasis.....	22	18	3	1
Ascariasis.....	7	0	4	3
Plague.....	3	3	0	0
Rabies.....	3	1	0	2
Chagas' disease.....	3	0	0	3
Hydatid disease.....	3	3	0	0
Histoplasmosis.....	3	3	0	0
Cysticercosis.....	2	1	1	0
Schistosomiasis.....	1	1	0	0
Actinomycosis.....	1	0	0	1
Total.....	1,178	784	210	184

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# AN ATTEMPT AT ACTIVE IMMUNIZATION WITH PLASMODIUM VIVAX KILLED IN VIVO\*

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It is now well known that convalescence from an untreated attack of vivax malaria is in part at least attributable to the acquirement of a very potent immunity to the strain of parasite which induced the attack, and we have shown (Boyd and Kitchen, 1943) that the level of immunity in such convalescents can be still further raised by a series of subsequent reinoculations with the homologous parasite. Indeed a level may be attained where the individual is able to withstand and promptly destroy doses of parasites many million times greater than the minimal number necessary to infect a fully susceptible person, and may with propriety then be regarded as hyperimmune. One of us has also discussed the criteria that may be used for the interpretation of the susceptibility status of persons naturally inoculated with vivax malaria (Boyd, 1942), with special reference to reactions indicative of homologous or heterologous immunity.

In view of these circumstances, it is important to ascertain whether there is any possibility that active immunization may be stimulated by the administration of killed parasites. The attainment of such an objective offers many practical difficulties, not the least of which arise from possible post-mortem alterations in the composition of the parasites, as well as the presence in the inocula of normal and parasitized erythrocytes or of material derived therefrom. Consideration of the problem led to the conclusion that the simplest approach might be afforded by the inoculation of graded doses of fresh parasitized blood into prepared patients, whose blood, prior to inoculation, had been saturated with a plasmodicidal drug. The parasites thus introduced, being fresh and living, would not have their composition altered in any fashion, and their prompt destruction in the saturated plasma of the recipient, should permit their bodies to exercise to the maximum any potential antigenic properties they might exert. Our own experience (Boyd, 1943) confirms the well-known fact that vivax infections induced by trophozoites are readily eradicated by plasmodicidal drugs.

Two presumably susceptible patients (B-3104 and B-3106) were, under the conditions described below, given three full therapeutic courses of quinacrine hydrochloride, the first beginning on June 13, 1945, the second on August 6, 1945, and the third on September 30, 1945. On the first day the patients received a total of 0.4 grams of the drug in divided doses, on the second day a total of 0.6 grams similarly given, and for ten days thereafter 0.3 grams in daily undivided doses, until they had received a total of 4.0 grams. On the fourth day of each course they were intravenously inoculated with blood con-

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taining living parasites of the McCoy strain of *Plasmodium vivax*, as shown in the following schedule:

PATIENT.....	B-3104			B-3106		
	1st	2nd	3rd	1st	2nd	3rd
Course						
Total gm. quinacrine administered before inoculation.....	1.4	1.4	1.4	1.4	1.4	1.4
Donor inoculum, patient no...	635	639	648	635	639	648
Volume heparinized blood administered intravenously (cc.).....	5.0	8.0	35	5	8.0	30
Trophozoites <i>P. vivax</i> (McCoy) in inoculum in millions. ....	79	109	263	79	109	225
Date inoculated. ....	6/16/45	8/9/45	10/3/45	6/16/45	8/9/45	10/3/46
Date of observation of parasites						
1st.....	6/16/45	neg.	neg.	6/16/45	neg.	10/4/46
last.....	6/17/45	neg.	neg.	6/17/45	neg.	10/4/46
Quinacrine level ( $\mu\text{g./L}$ ) plasma						
a) prior to inoculation on 6/16.....	>120	>120	>120	>80	>120	>120
b) subsequent to inoculation on 6/17.....	>120	>120	>80	>60	>120	>120
Total gm. quinacrine given subsequent to inoculation.....	2.6	2.6	2.6	2.6	2.6	2.6

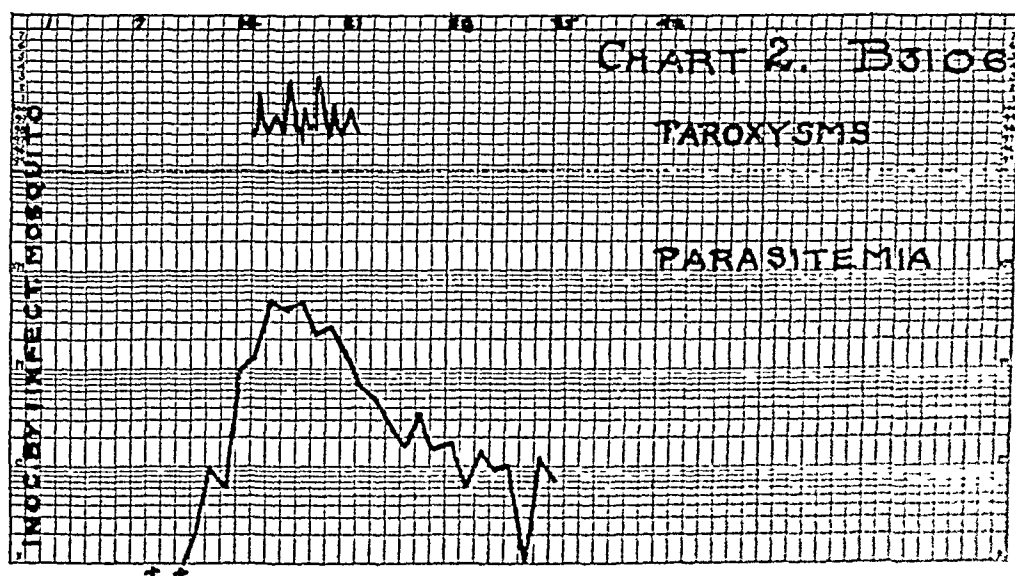
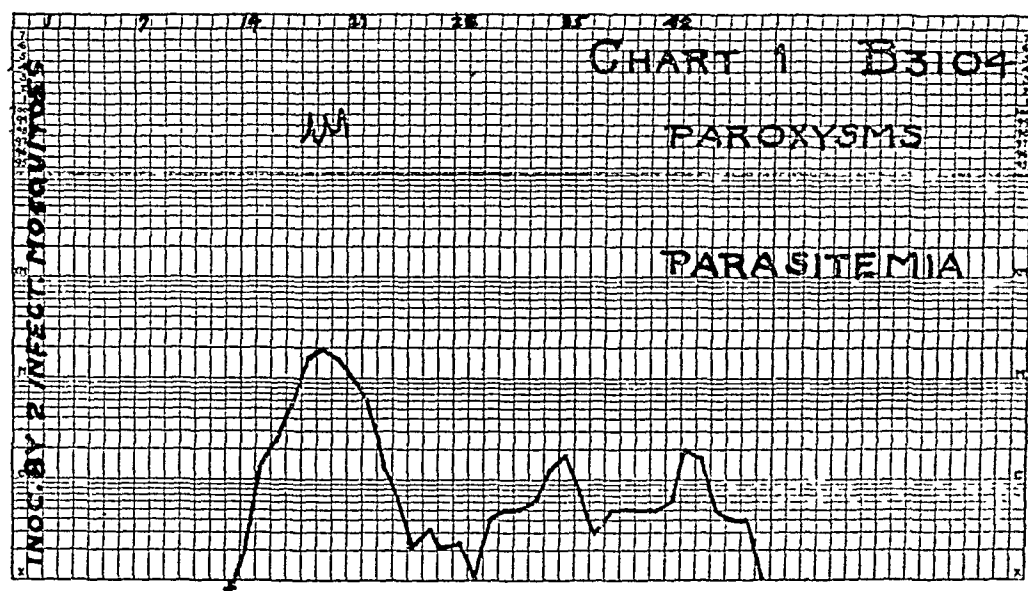
It should be noted that the parasites of the several inocula were at the most never detected for more than 24 hours following the inoculation and that the several doses of parasites were introduced when high plasma levels of quinacrine prevailed. Both patients enjoyed uninterrupted good health, and daily blood examinations revealed parasites only on the indicated occasions.

Since it is obviously necessary to await complete or nearly complete excretion of the accumulated quinacrine before ascertaining the effect of these inoculations subsequent determinations of the plasma levels of the drug were made as follows:

DATE	PATIENT	
	B-3104	B-3106
11/29/45	40 < $\mu\text{g./L}$	40 < $\mu\text{g./L}$
1/ 2/46	below 40 $\mu\text{g./L}$	below 40 $\mu\text{g./L}$
2/19/46	5 $\mu\text{g./L}$ *	8 $\mu\text{g./L}$ *
4/ 2/46	1.6 $\mu\text{g./L}$ *	—
4/28/46	—	0.9 $\mu\text{g./L}$ *

\* Determination made in the laboratory of Dr. David P. Earle, Goldwater Memorial Hospital, New York City. The determinations of 4/2 and 4/28 may, according to Dr. Earle, represent either a trace of the drug or a high blank reading.

Our own crude facilities for the determination of quinacrine in plasma not permitting its estimation in amounts below 40 micrograms per liter, a request for assistance was made to Dr. David P. Earle, Jr., by whose courtesy the last determinations were made in the Goldwater Memorial Hospital, New York, City.



It was concluded that the small residual amount of quinacrine present in patient B-3104 might be ignored. On 4/2/46 he was inoculated by the application of two *A. quadrimaculatus*, in the salivary glands of which, on subsequent dissection, abundant sporozoites were found. These were infected with the McCoy strain of *P. vivax*. Parasites were first seen in the blood smear of 4/16/46

and a clinical onset was experienced on 4/21/46, which initiated a series of three quotidian paroxysms which terminated spontaneously. The course of his parasitemia and clinical reaction is shown in chart 1.

Patient B-3106 was inoculated with the McCoy strain of *P. vivax* on 4/19/46, by the application of a single *A. quadrimaculatus*, in the salivary glands of which abundant sporozoites were found on subsequent dissection. Parasites were first found in the blood smear of 4/28/46, and the clinical onset on 5/5/46 initiated a series of seven quotidian paroxysms, with marked difference in the severity of the paroxysms produced by one brood of the parasites. They terminated spontaneously. The course of his parasitemia and clinical activity is shown in chart 2.

Consideration of these charts brings out the following points: The infections become patent after normal intervals of prepatency, but both patients exhibited a high pyrogenic threshold, in excess of 1,000 parasites per cmm.

The maximum densities attained by the parasitemias were low (2,000; less than 5,000) with a subsequent rapid decline. In both patients the clinical activity was limited to the period when the parasitemia exceeded 1,000 per cmm. In patient B-3104 the latent infection was microscopically patent for four weeks, and in the other for five weeks following termination of clinical activity.

*Conclusions.*—Assuming that these patients were originally fully susceptible they did not, on final inoculation with living sporozoites following excretion of the quinacrine accumulated during the course of their immunization inoculations, prove refractory to inoculation, but on the other hand, developed an infection. However, the course of their infection exhibited certain very definite characteristics of immunity, namely: (1) the high pyrogenic level, (2) the low maximum density attained by the parasitemia, (3) the transient character of the maximum, (4) the rapid spontaneous decline in the parasitemia, and (5) the short clinical attack. While elsewhere (Boyd, 1942) such a reaction has been observed following known reinoculation with a heterologous strain of *P. vivax* rather than with the homologous strain, it would appear that in these instances the patients did acquire an appreciable immunity following the intravenous administration of living parasites when their plasma was saturated with quinacrine.

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# STUDIES ON ATABRINE SUPPRESSION OF MALARIA

## II. AN EVALUATION OF ATABRINE SUPPRESSION IN THE FIELD

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Since the value of a drug as a suppressive of malaria depends on the total amount of malaria which it suppresses, it is obvious that the rate of clinical attacks in troops on irregular suppression tells us little about the efficacy of the drug. Both the actual amount of clinical malaria and an estimate of the expected attack rate were suppression not in effect must be available. Since it is often not feasible to take troops off of suppression to find out the amount of malaria suppressed, it follows that a method or combination of methods for determining just what is going on during suppression would be of great value. We believe that by careful parasite surveys of troops it is possible to determine some of the factors in operation.

### DEVELOPMENT OF METHOD

During a routine study of the treatment of acute attacks of both vivax and falciparum malaria it was noted that a few parasites (ring or comma-shaped forms) could often be found reappearing in the blood late in the course of therapy. (1) They were usually not accompanied by a rise in temperature, were often less than 1/500 w.b.c. and always less than 4/500 w.b.c. The more attacks of fever a man had the less likely he was to show this phenomenon. Atabrine levels were well above 30 gamma per liter in all cases.

Similar studies were then made on other men who had not had a clinical attack for some weeks and who were under rigidly supervised atabrine suppression. A highly infected group was chosen for this, (80% relapsing 6 weeks after stopping suppression), and in one company 14% were shown to be positive on one smear. Again the transient parasitemia was unrelated to the atabrine levels, which were normal for suppressive levels (fig. 1).

It also appeared that previous sensitization of the phagocytic system played some part in the phenomenon, for "Co. I" which produced this high percentage of positive smears was composed of men who had had only a few previous attacks, while the men in Company A and E with a low % pos. had had many previous attacks (table 1.)

From this we have assumed that the percentage of positive smears which can be found in troops who have not previously had much clinical malaria is a rough measure of the amount of infection.

Thus on surveys, although positive smears may be obtained in clinically well

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individuals on adequate suppression, there were few with more than 5 per 500 w.b.c. We may assume then that parasite densities above this level indicate multiplication in the blood and therefore inadequate suppression.

If as the accompanying table shows, any considerable proportion of those who are positive have five or more parasites per 500 w.b.c., then it is assumed that these men have not been effectively suppressed even if they happen to be asymptomatic.

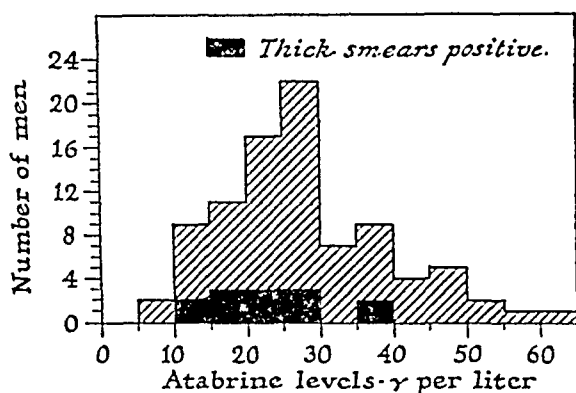


FIG. 1. PLASMA ATABRINE LEVELS IN MEN SHOWING POSITIVE THICK SMEARS COMPARED WITH NEGATIVE SMEARS

TABLE 1

*Effect of immunity on incidence of positive smears*

	% WITH 3 OR MORE ATTACKS	NO SMEARS	NO. POS.	% POS.
Co. A	100%	99	0	0.0
Co. E	90%	250	2	0.8 ± .5
Co. I*	25%	145	21	14.5 ± 2.9

\* 42% of these men had only 1 attack.

#### METHOD

The rates of infection were determined by a careful examination of Giemsa stained thick smears by one of the officers of the group. Every positive slide was checked by another officer. Since these surveys were made by four experienced men working closely together the procedure was fairly standard. Positive slides are expressed as parasites per 500 w.b.c. which means that a minimum of 0.1 c.mm. blood was examined. Most negative slides were further examined for another five minutes. The estimate of suppression included in the table is that of the base malariologist who inspected the atabrine roster during the administration of the drug. Malaria rates are for the three weeks preceding and one week following the parasite survey, and are expressed as cases/1000 men/yr.

The results of the surveys are presented in table 2. They show fair correlation between the actual malaria rate, the estimate of efficiency of atabrine administration and the results of our surveys.

Such surveys as these have definite limitations. First, they are of little value in telling us how conscientiously atabrine is being taken if there is not much malaria in the outfit to start with. Secondly, an outfit may be heavily infected and because the men have had many clinical attacks few parasites would be apparent with good suppression. Yet within these limitations the method emphasizes the fact that a given malaria rate may be produced by a variety of conditions.

With these considerations in mind we have attempted to estimate the efficacy of atabrine suppression during jungle combat, the period when it is most severely tested.

#### SUPPRESSION OF MALARIA IN COMBAT

There is no longer any doubt that atabrine is highly effective in the suppression of malaria even in combat, but there has been no unanimity of opinion as to just how much malaria we might expect under suppression.

TABLE 2  
*Parasite rates in "infected" troops under suppression*

OUTFIT	TIME IN MALARIOUS AREA	MALARIA RATE	SUPPRESSION	NO.	% POS.	% OF POS. MORE THAN 5 PARA.
Port Bn.	8 mos.	442	Very poor	174	17.2	53
St. Hospital	5 mos.	287	Fair	253	7.1	11
Eng. Bn.		53	Fair	151	2.0	0.0
Inf. Reg.	2 mos.	70	Fair (under combat)	184	2.2	0.0
6th Army trainees	8 mos.	0	Excellent	145	14.5	0.0

Three factors make the suppression of malaria during jungle warfare more difficult than in base areas. First, such outfits usually carry a heavier load of previous infection and are continually being reinfected. Secondly, supervision of atabrine rosters becomes more difficult and often impossible during combat. Finally, the stress of living conditions may stimulate the outbreak of clinical malaria in the occasional individual who is unprotected by 0.1 gm. per day even though there is no apparent effect of exercise on atabrine metabolism (2).

A study of the 126th Infantry Regiment of the 32nd Division was undertaken because all of the above difficulties of suppression obtained. So conclusions concerning the efficacy of suppression in these troops are applicable to less heavily infected troops under less severe conditions. This regiment was heavily infected during the Buna campaigns. Although there were many replacements in the ensuing eighteen months, some infection remained. The amount of recent infection may be judged by the fact that at the time of the study 40% of all the infections were falciparum and 50% were first attacks. Although the rate of anopheles infection was low (none positive of 122 dissections) many men were infected—this because the men were constantly exposed to many mosquitoes in pill boxes

and foxholes. When this study was started the regiment had been in severe jungle combat for the previous two months and in light combat during the four preceding months. A high proportion (10%) of the replacements by this time showed parasites despite suppression and lack of symptoms. They were taking 1.0 gm. atabrine/week, divided into two or three doses.

The previous study of break-throughs (2) which occur in base areas has shown that the problem of suppression of malaria in service troops is mainly one of

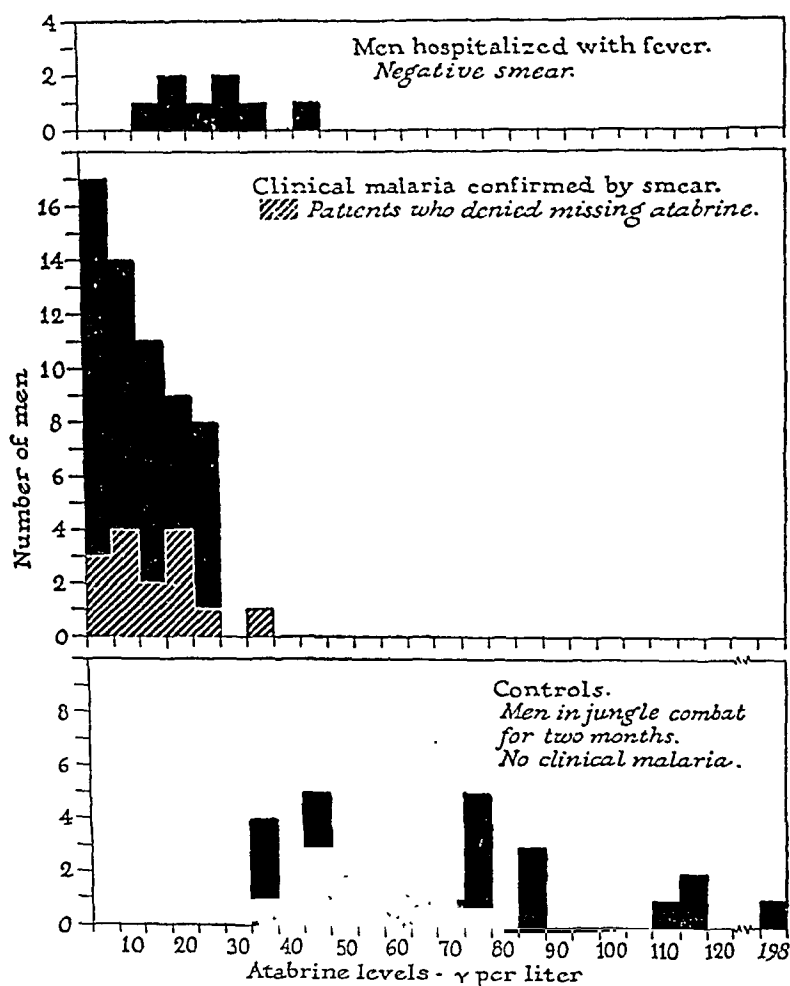


FIG. 2. ATABRINE LEVELS UNDER COMBAT CONDITIONS

Suppressive regime: 0.5 g. 2 times per week for 2 months, subsequently 0.2 g. per day administration and that cases rarely develop in men who have conscientiously taken 0.1 gm. atabrine/day. Plasma levels of men developing malaria when in combat (fig. 2) are no higher than in those who relapse in a non-malarious area. Thus the fundamental problem of suppression in combat does not differ from the problem elsewhere.

In this study the controls had higher atabrine levels (fig. 1) than those in other studies since more atabrine was taken each week. No study similar to this has been carried out with 0.6 gm. or 0.7 gm. atabrine/week in combat, and we cannot



conclude that 0.1 gram atabrine taken once a day would not suppress malaria in combat. One case of malaria had a level about 30 gamma per liter; this man had taken his usual 5 pills the previous night and it is likely that had he been able to maintain this level he would have cured himself. This emphasizes the fact that the atabrine level obtained when the patient comes to the hospital may not be the same as the level which existed at the time of the developing parasitemia.

Cases of malaria among troops on suppression may be due either to failure to take the prescribed dose or to an abnormal metabolism of the drug. A low atabrine level does not necessarily mean that the patient has not been taking the prescribed dose. Thus we can obtain an estimate of the percentage of true break-throughs only by a series of careful histories with atabrine level studies. These were carried out as follows: At the time of bleeding for atabrine levels, the sergeant who took the blood questioned each man carefully. The next day one of us again independently questioned the patient. It often took 15 or 20 minutes of careful questioning before he admitted to any break in regime at all. Then it was often discovered that the regime had been very inadequate. Two patients told the sergeant that they had missed atabrine but failed to tell the medical officer. Sample histories are given in the accompanying table. Of 72 cases studied on admission to the hospital the following results were obtained.

Vomiting and/or missing atabrine.....	43
Quinine instead of atabrine.....	5
Diagnosis not confirmed by smear.....	8
Denied missing atabrine.....	16
Total.....	72

An individual was not considered to be missing or vomiting enough atabrine to make his suppression inadequate unless he had missed at least one dose of 0.5 gm. the previous week and one or more the week before that. Probably a few of these 16 men denied missing atabrine for fear of punishment. We concluded that over 75% of the cases who came to the hospital could be eliminated from our consideration of true break-throughs.

This does not of course tell us how many of these men would have malaria were they not suppressed. We have tried to get at this indirectly. It was found on previous studies of vivax malaria that heavily infected men might have as high as 14% positive smears even though they were asymptomatic and under excellent suppression. Of this group of more than a hundred men when taken off suppression, 80% developed relapses in 8 weeks.

Studies of smears taken from the men in the 126th Infantry Reg. while in combat who had had few or no previous attacks of malaria showed 12 of 114 positive, or 10%. This rate of parasitemia in well-suppressed soldiers (see fig. 2) might well indicate that about 50% would relapse in two months if unsuppressed.

The actual malaria rate which was carefully checked was 140/1000 men/year. A maximum of 25% of this or 35/1000/year was due to valid break-throughs. If with no suppression 50% had developed malaria in two months we would have had an attack rate of 3000/1000/year. Thus only about 35 of 3000, or 1 out of 100 possible attacks did develop despite atabrine. If we assume that the 10%

TABLE 3  
Studies on individual breakthroughs

No.	SUPPRESSION		MALARIA		
	1st History	2nd History	No. attacks	Ata-brine level	Parasites per 500 w.b.c.
1	10/wk. Occasional vomiting	Because of vomiting pt. cut down to 3 at each dose. Also missed. Thus got 8 in last 2 weeks.	0	0	P. falciparum 3,580 rings
2	10/wk.	Taken under supervision. No vomiting. Last dose 5 days previously.	0	9	P. vivax 300 ame-boid
3		Because of vomiting 5 pills 2 times/wk., started 4 wks. ago taking one pill/day, sometimes atabrine, some-times quinine. 5 days before hospitalization took 15 at. then took "some" quinine.	0	9	P. falciparum 2,650 rings
4		While in camp took 3 pills 3/wk. and kept these down. When on line had to take 5 at a time and was "so burnt up" that he took none for 6 wks. Last week took 2/day.	2	8	P. vivax 110 rings
5	10/wk.	Never misses atabrine. "Too smart not to take it."	1	15	P. vivax 1,900 troph.
6	10/wk.	While on push against Japs atabrine became very irregular.	0	16	P. sp. 450 rings
7	10/wk. Occa-sional vomiting	Missed 2 doses before this illness. Previous study of this pt. 1 yr. before showed that he developed low levels on intensive therapy and was barely suppressed on .1 gm./day. Chills and fever for 2 wk.	6	0	P. vivax 180 ameboids
8	10/wk.	Takes 3 pills 2/wk. and gives left over to his buddies.		9	P. vivax 6 rings

TABLE 3—Continued

NO.	SUPPRESSION		MALARIA		
	1st History	2nd History	No. attacks	Ata-brine level	Parasites per 500 w.b.c.
9	Missed	Caught in a retreat and failed to get atabrine for 7-10 days. Started 1/day 2 days ago.		14	<i>P. falciparum</i> 200 rings
10	10/wk.	Does not think he missed although on a drive into enemy territory in past wk.		4	<i>P. falciparum</i> 75 rings
11	8/wk. Missed while in hills on patrol	Taking 4 pills 2/wk. Did not miss or vomit. Neg. smear at first.		8	<i>P. falciparum</i> 11,-800 rings

positive smear index indicates an attack rate only half of the above, we still have only 2 out of a possible 100 attacks or 98% protection.

## SUMMARY

An evaluation of atabrine suppression under field and combat conditions is attempted. The malaria rate in itself is of little value since the total amount of malaria to be suppressed is unknown.

The percentage of men showing positive smears (very few parasites) when on good suppression (checked by atabrine levels) is assumed to be roughly indicative of the amount of malaria in this group of men. An index of 14% positive was obtained in a group of whom 80% relapsed with attacks of vivax malaria within two months after stopping atabrine. This relationship is true only if the smears are made on individuals who have had few or no previous attacks of clinical malaria.

A combination of careful history taking, determination of atabrine levels, and examination of smears showed that at least 98% of the men in strenuous combat were protected by 1.0 gm. atabrine per week. This was usually taken in 2 doses of 0.5 gm. each. No data on 0.6 gm./wk. from troops during strenuous combat is available. Sample histories showing the difficulties of administration are given.

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# RELAPSING FEVER ON THE ISTHMUS OF PANAMA

## REPORT OF 106 CASES

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Some reports have been published with respect to the clinical aspect of relapsing fever on the Isthmus of Panama since the first reports of Darling (1-2) and of Connor (3), that cover the diagnosed cases until 1917. Among such reports are those of Bates (4), who described for the first time the transmission of the disease experimentally from animal to man; of Clark (5), who also transmitted the disease to man experimentally; of Carrizo (6), who reported four cases, one of which was hospitalized in Santo Tomás; of Nicosia (7), who reported one case hospitalized in Santo Tomás; and of Cohen (8), who reported six cases hospitalized in Gorgas Hospital on the Canal Zone. In this paper we discuss the clinical aspect of relapsing fever on the Isthmus of Panama, with various references to its etiology, epidemiology, prognosis and treatment, based on the observations of the patients we had the opportunity to attend in our clinic and records of the other 32 cases diagnosed in this hospital, from 1927 to 1944; and of those of 72 patients from Gorgas Hospital, on the Canal Zone, from 1907 to 1944 inclusive, a total of 106 observations.

*Etiology:* It seems beyond all doubt that recurrent fever on the Isthmus of Panama is produced by a new strain of *Spirochaeta recurrentis*, for which the name of *Spirochaeta neotropicalis* (10) has been recommended. The immunologic investigations (11) show that it is a distinct strain. The study in conjunction with the bibliography strengthens our belief that it is preferable to consider the described spirochaete of Panama as a new strain rather than as a new species of *Spirochaeta recurrentis* (12), by adaptation in its local animal vectors.

*Epidemiology:* In the study of relapsing fever on the Isthmus of Panama from the epidemiologic standpoint one must consider the number of wild animal carriers of the disease (5, 9, 13 to 15) and the susceptibility of various mammals to the disease (1, 16, 17); the quantity of *Ornithodoros venezuelensis* and *talaje*, many being infected, which infest the farm and urban dwellings, in the same order of frequency, and are the transmitting agents of the disease (4, 5, 9, 13, 16); its life cycle (13 to 16) and also that the disease can be acquired congenitally (4-5); finally, that in the central portion of the Isthmus is located the Canal Zone, with its American population.

We do not wish to dwell upon the three primary considerations previously mentioned, because the publications referred to in the bibliography are sufficient from this standpoint. We only wish to emphasize with regard to the immigration of Americans that this gives us a large population with no immunity, which works in a region rich in infected ticks where one can easily acquire the disease that develops with characteristics generally not observed in our native farmer, who has already acquired some degree of immunity.

A study of the cases show that the *incidence* of relapsing fever is relatively low, with an average of 0.11 per year and per 1000 hospitalizations.

It does not appear that *age* influences the incidence of the disease; our statistics show patients with ages varying between 13 months and 50 years. However, 82.5% of the total number were between 11 and 40 years of age corresponding to the greatest working activity.

No specific *nationality* of people is immune to relapsing fever; the major incidence occurred in Panamanians, with Americans being next in order of frequency; however, in relation to the total number of the one and the other nationality who inhabit the Isthmus, there is a greater incidence among the Americans, because of their lack of immunity.

There seems to be no predilection for *race*, since white people as well as mestizos and negroes have been infected with relapsing fever in nearly equal frequency.

The incidence has been greater in males than in females, and during the dry months rather than during the rainy season, much to the contrary of reports in text-books (18 to 20).

There is no area in the interior of the Republic from which no report has been made of this disease. Our investigations, show that in the neighboring republics of Colombia and Ecuador the same disease exists.

*Symptomatology:* From the reports of 93 medical histories we make our conclusions according to the following topics; *subjective symptoms, physical examination and laboratory examinations.*

1. *Subjective symptoms:* There are reports of cephalgia and anorexia during the incubation period; there are no complete nor adequate records concerning this period, except for the author's data; however, it is interesting to note that the majority of text-books, likewise, have very little information in regard to this aspect. It does not help to make a very easy diagnosis of relapsing fever; but as has been shown in other diseases in this one there is no exception in respect to its premonitory symptoms of the incubation period which, as reported by Bates, Dunn and St. John (4), is of 6 to 9 days' duration.

The onset of the period of invasion is revealed by fever and violent chills. The chills were violent in 71.6% of the total and in 97.9% of the cases reported; in only two histories was there denial of the existence of chills. In 23% of the total cases the chills recurred daily with each rise in temperature, at the end of each remission or of the period of apyrexia; in 16.8% of the cases the chills were manifested as a sensation of continuous chilliness, accompanied by psychic depression.

The temperature followed a course of repeated febrile periods or cycles in successive form; 56.8% of the patients presented a secondary febrile cycle; 17.8%, a tertiary cycle; 6.3%, a fourth cycle; and 2.1%, a fifth cycle. We cannot establish any average for the number of febrile cycles in our patients because in nearly all of them treatment was initiated as soon as a definite diagnosis was made by observing the *Spirochaeta neotropicalis* in the circulating blood; but this gives an idea as to how many cycles may be expected and at the same time permits one to refer it to one or the other of the two classic forms of the disease; the one, transmitted by ticks and the other transmitted by lice.

The primary cycle lasted from 2 to 9 days, with an average of 4.9 days; the secondary cycle, in 49 clinical histories, lasted between 1-5 days, averaging 2.5 days; the tertiary, among 16 histories, lasted 1-4 days, averaging 1.5 days; the fourth cycle, among 9 case reports, lasted 1-4 days, averaging 1.4 days; and the fifth cycle in two cases lasted 1-2 days, with an average duration of 1.5 days. With each successive cycle, the average duration of the febrile cycle decreased.

The fever was of a remittent type in 78.9% of the total reports; intermittent, in 9.45%; remittent in one of its cycles or intermittent and vice versa in the following cycle, in 6.3%; in 6.3% of the cases the type of fever was unknown because the patients were observed and treated during the febrile period.

The average temperature per cycle decreased from 103.8 degree F. in the primary, to 103.4, 102.8, 101.9 and 101.6 degrees F. in the succeeding cycles.

Similar observations were made regarding the maximum temperature per cycle, which was 106 degrees F. in the primary cycle and gradually decreased to 105.6, 105, 102.5 and 102.4 in the following cycles. A study of the daily temperature per cycle shows that in the primary cycle the average daily temperatures were 103.8, 102.9, 102.7 and 103 degrees F. respectively, with the temperature higher at the onset and termination of the cycle; similar observations were made in those cases in which the primary febrile cycle was of a longer duration than the one indicated above. It is noted that in the secondary cycle, with average daily temperatures of 102.9, 102.1 and 102.6 degrees F., the maximum temperature occurred at the onset and crisis of the cycle. The histories of cases with three to five cycles are not numerous enough to make definite conclusions on the average daily temperature.

Only four patients were delirious with the maximum temperature in the primary cycle; none presented convulsions, coma, nor meningeal symptoms.

*Cephalgia* accompanied the temperature (positive in 84.2% of the cases), being frontal in type especially (a few being of a skull-cap type, fronto-occipital, fronto-temporo-occipital), which required the use of strong and repeated doses of analgesics, and was considered the most constant symptom and chief cause of psychic depression in the patients; these headaches were recalled with dread and described as penetrating.

The fever at the end of each febrile cycle always fell by rapid crisis, accompanied by profuse perspiration.

With the drop in temperature at the end of each febrile cycle an interval of apyrexia followed; in our series of observations it is noted, in general, that the total duration and average of each period of apyrexia decreased from the first to the fourth period, in relation to 6.9, 6, 4.7 and 3 days respectively. With the apyrexia the patients feel well, although in a few instances moderate asthenia persisted following the febrile cycles as the only sequel of the disease.

Among other subjective symptoms reported in the clinical histories, pain in various regions of the body was a common complaint, being reported in 34.65% of the cases as generalized pain; in the dorsum of the thorax in 6.3%; in the legs, articulations and muscles in 2.1% respectively; in the left hemithorax, lumbar region and spinal column in 1.5% respectively. Only eight patients, 8.4%,

reported abdominal pain, diffuse in the majority of cases, moderate and without concomitant signs of peritoneal involvement.

In 46.3% of the cases there was a report of nausea, and vomiting in 41%; only eight patients denied having any nausea, and only 12 denied vomiting. The vomitus always consisted of food material and gastric juices; bile was present in only four cases; no hematemesis was reported; none of the vomiting was of an incoercible type.

Eleven patients complained of having a cough; four with naso-pharyngeal catarrh. In only 26 cases was there any report of anorexia; twenty two reported asthenia; 7 reported insomnia. Although these values are small, they are significant since all cases reported were positive.

In only two patients was there any complaint of discomfort with urination, without evidence of cystitis or urethritis, and this complaint ceased with the fall in temperature.

The chief disturbance associated with the intestinal tract was an alteration in frequency of bowel movements, manifested as constipation in 8.4% of the cases, mucous diarrhea in 7.35%, and one patient presented a dysenteric syndrome, without active *Endamoeba histolytica* present in the stools. None of these patients required special treatment to correct the diarrhea; there are no reports on the course of the patients with constipation.

2. *Physical examination:* In only two patients was there any report of some cardiac lesion; we do not think that these organo-functional disturbances were due to any toxic action of the spirochaete of relapsing fever.

The blood pressure averaged, in general, around 110-70 mm. Hg.; in male patients less than fourteen years of age, as in adults with hypochromic microcytic anemia due to uncinariasis, the differential was as small as 20 mm. Hg.

The pulse was always regular, full and equal, with the exception of one patient; there was an increase of 15 beats per minute on the average per degree Centigrade of fever; in three experimental cases of Clark, the average increase in pulse rate was 12 beats per minute per degree Centigrade of fever. In general, the pulse rate was proportional to the temperature.

There was no particular participation of the pulmonary system in the cases of relapsing fever attended in Gorgas and Santo Tomás Hospitals; however, in ten cases there were various pulmonary manifestations (chronic bronchitis in eight patients; bronchopneumonia in one; congestion of the right base in another case) which may be considered only as complications of the disease.

Abdominal palpation revealed some sensitivity, especially in the right hypochondrium (5 cases) and in the epigastrium (4 cases); but there was nothing specific which might be considered as pathognomonic.

We think we are right in believing that in 25.2% of the cases there is no doubt that the splenomegaly was due to the action of the spirochaete of relapsing fever; the congestion and sensitivity (there are no other data) has been confirmed; but there is no explanation in regard to the other patients, a total of 22.1%, who presented splenomegaly "without pain" because there is no further report on these cases in respect to their final course, and we know that some patients had had malaria and lived in areas with endemic malaria.



As in the previous paragraph, we believe that 18 cases, 18.9%, of hepatomegaly with sensitivity were due to the effect of the spirochaete of relapsing fever; but we cannot attribute the other cases of hepatomegaly without pain as being due to this cause, since there are multiple factors in the tropics capable of producing hepatic cirrhosis and hepatomegaly.

Only 5.25% of all the patients presented icterus, which was always accompanied by hepatomegaly and sensitivity on palpation of the liver.

*Insect bites* were observed on the extremities, thorax and abdomen of ten patients; herpes labialis in two cases; and in two others, 2.1%, during primary and tertiary febrile periods there was a macular erythema on the thorax and abdomen, without relation to the therapy used. There are reports of congestion of the face and pallor of the remaining portions of the body during the febrile stage, followed by blanching of the face at the crisis of the fever.

In 10.5% of the patients there was pharyngeal congestion; tonsillar congestion in 4.2%; pharyngeal and tonsillar congestion in 6.3%.

In only 4.2% of the cases was there any sub-acute submaxillary adenitis; all of these had tonsillar congestion. There were reports of chronic evolution of adenitis in many other cases. It is doubtful that the lymph nodes participate in the usual course of relapsing fever on the Isthmus of Panama.

Three patients presented meningismus, with slight rigidity of the neck; there was no other symptom of meningeal irritation.

3. *Laboratory Examinations:* The erythrocyte counts of the patients with relapsing fever showed a very slight anemia, never less than 4,000,000 per cm. There were many cases in which the red cell count was low but this was due to *uncinaria* infestation.

The leucocyte count averaged around 8,500 per cm. with a maximum leucocytosis of 16,000 and a minimum of 3,000 per cm. The moderate leucocytosis associated with relapsing fever is mentioned in various text-books.

The differential leucocyte count, in general, showed nothing specific nor pathognomonic; there were cases with neutropenia and others with moderate neutrophilia; lymphopenia and lymphocytosis; moderate eosinophilia and monocytosis.

The hemaglobin concentration averaged 70% (Tallqvist), varying between 25-90%; all those cases with a hemaglobin content below the average had an *uncinaria* infestation.

Albuminuria was encountered in only two patients; the remainder of the urine examinations were normal. There was practically no renal pathology in our patients with relapsing fever.

Without relation to the time of extraction of blood (febrile or afebrile period), in only one patient was the *Kahn test* strongly positive (4 plus). We have no information on the past luetic history of this patient nor any repeated examination reports to judge the significance of this reaction; however, if the test had been negative before the onset of the fever, this report shows that the percentage of false reactions (4 plus) during relapsing fever in Panama is exceedingly low, in frank contrast to the observations of some investigators who give a higher percentage of 8%. There were seven cases of false reactions with 2 plus and

two cases with 1 plus, which we have not included in the total of false positive tests, because of their very moderate flocculation.

Examination of spinal fluid after the febrile cycle in two patients with arsenical therapy already initiated showed no abnormality in color, pressure, cellular and chemical content, nor were any spirochaetes encountered. The Wassermann reaction was negative in both cases.

An infection frequently encountered in our rural population is intestinal parasitism, which was positive in 29.4% of all our cases of relapsing fever; uncinariasis was encountered in the majority of cases although mixed infections were observed (*Ascaris*, *Trichuris*, *Strongyloides*).

Another common infection, which can complicate any other pathogenic agent in our territory, is *paludism*, being positive in four of our patients. One of these cases was infected with *Plasmodium malariae* (which is very rare in the Isthmus) and this infection is the more interesting of the two common ones associated with relapsing fever, since this also is encountered in the circulating blood.

*Treatment:* We can summarize the therapy of relapsing fever, as noted in the clinical records, as follows:

1. When large doses of arsenicals were injected or when the same or smaller doses were injected at the end of the primary febrile cycle, when the crisis was suspected, the majority of cases had immediate reaction after the injection.

2. New febrile cycles were observed more frequently when the arsenical was given intramuscularly than intravenously.

3. Moderate doses (0.45 gm. of neosalvarsan, for example, rather than large doses such as 0.6 gm.) in adults injected during the afebrile stage or during the febrile crisis were sufficient to prevent recurrences of fever, especially when the doses were repeated; in none of these cases was there any arsenical reaction.

We do not think that large doses of arsenicals (20) should be used in the treatment of relapsing fever; and (19) that as soon as a definite diagnosis can be made (during the febrile stage) the arsenical at hand should be injected, unless a febrile crisis has already commenced or if a crisis is imminent, when it is better to wait several hours until the crisis has terminated, before administering the arsenical. However, it is not wise to recommend a dose as large as 0.9 gm. of neosalvarsan, injected at one time; nor is it wise to wait, in the period of apyrexia, for a new febrile stage to give another injection. Repeated moderate doses (as 0.45 gm. of neosalvarsan or neoarsphenamine) have been observed to be more beneficial in preventing further relapses, with the initiation of therapy during pyrexia (as recommended by Manson-Bahr), or apyrexia as soon as a definite diagnosis is made with the benefit, in the majority of cases, of avoiding the recurrence of the fever; our observations also show that there is a relationship between the arsenical used and the course of the disease and its febrile curve.

We cannot say that one arsenical is inferior in action against the spirochaete of relapsing fever because in one or two patients its action was not dramatic. We do not refer to those cases in which insufficient doses were used but precisely to those cases in which sufficient dose was given, because there are numerous

observations for the same arsenical, such as novarsenobenzol and neosalvarsan, for example, with contradictory results. It is advised in such a case to change the type of arsenical to be used.

It is important to remember that in those cases after the first reaction, there were no similar manifestations afterwards, even though similar or larger doses of novarsenobenzol, neosalvarsan and mapharsen were used during the afebrile period.

Finally, we wish to mention that the majority of patients were treated with neosalvarsan and that with this arsenical the minimum febrile relapses and reactions were observed.

#### CONCLUSION

1. The laboratory and clinics have confirmed the diagnosis of relapsing fever on the Isthmus of Panama.

2. It appears that the etiologic agent, *Spirochaeta neotropicalis*, is a strain of *Spirochaeta recurrentis*.

3. Some wild animals are hosts of *Spirochaeta neotropicalis*.

4. *Ornithodoros venezuelensis* is the principal vector of the spirochaete which can be transmitted also by *Ornithodoros talaje* from animal to man.

5. The incidence of the disease on the Isthmus of Panama is very low, no greater than 0.11 per 1000 hospitalizations. It is observed chiefly between the ages of 14 and 36 years (in relation to the years of major activity in the fields), without relation to nationality or race, and in our farmers, with no relation to sex. Its major incidence is during the dry months of the year, in women as well as in men. There is no specific regional focus of origin; apparently the disease exists in all areas of the republic.

6. In regard to symptoms it is impossible to distinguish this disease from the clinical type described in San Francisco (21). With the exception that we have not encountered the rash nor the splenomegaly with the frequency of those cases reported in Texas and that the new febrile cycles are less severe than the primary or previous ones, our cases are identical to those cases of relapsing fever described in Texas (Kemp, Moursund and Wright, 1935) in respect to the symptomatology. Our cases differ from those of Central Africa in that the fever decreases in our clinical type from the primary to the secondary, third and fourth febrile cycles; also in our cases there is no significant frequency of diarrhea nor of a dysenteric syndrome (19) as described in the above mentioned patients; and the pulmonary complications (pneumonia and bronchitis) and changes in the blood picture (such as polymorphonuclear leucocytosis and aplastic anemia), have not been encountered with such frequency as described elsewhere.

7. According to the medical histories we have reviewed the prognosis in this disease is good; no deaths have been reported.

8. The only sequela has been asthenia, in a few cases.

9. With sufficient doses of an arsenical, injected intravenously (0.45 gm. of neosalvarsan or neoarsphenamine, repeated within 24-48 hours after the initial dose of 0.3 gm. for a patient weighing 55 kilograms, in the tropics) the results

have always been satisfactory. In several patients (4%), however, it was necessary to change the arsenical because of recurrence of the febrile stage. We do not feel that it is necessary to wait for the febrile stage to initiate or continue the treatment; it is advisable to commence with the therapy as soon as the diagnosis has been made, unless a febrile crisis is imminent, to avoid a recurrence of the febrile cycle or a post-injection crisis.

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# BIOLOGICAL STUDIES ON ENDAMOEBA HISTOLYTICA

## I. THE GROWTH CYCLE OF POPULATIONS IN A MIXED BACTERIAL FLORA<sup>1</sup>

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### INTRODUCTION

Knowledge concerning the biology of parasitic Protozoa has lagged far behind that of related free-living forms, owing to the necessity of first discovering suitable methods of cultivation *in vitro* and to the more rigorous experimental conditions required. Effective work on intestinal amoebae, for example, has been possible only within the past two decades, since Boeck and Drbohlav (1) initiated the first successful culture method for *Endamoeba histolytica* in the presence of a bacterial flora. During the intervening years additional culture media have been developed empirically for intestinal amoebae (especially *E. histolytica*), always with the condition that bacteria must be present: derivatives of hen's egg plus supplement (2, 3, 4); asparagin-agar plus horse-serum overlay (5); liver-infusion agar or Loeffler's dehydrated serum plus serum-saline overlay (6); beef-heart infusion (7); peptone-meat-extract infusion (8); etc.

A beginning has been made in the analysis of factors influencing the growth of *E. histolytica*. Dobell and Laidlaw's extensive study (2) included incomplete data on tolerance to various physicochemical conditions, influence of the bacterial flora, factors inducing encystation, etc. Snyder and Meleney have demonstrated that factors inducing excystation are simpler than those controlling growth (9), and the same authors (10) produced evidence to show that anaerobiosis and cholesterol are growth requirements. Chang has contributed careful studies of the relation of pH to encystation (11) and of the relation of oxidation-reduction potentials to growth and encystation (12). Rees and his co-workers have contributed the valuable technique of isolating *E. histolytica* with single strains of bacteria (13), and have been using such cultures to test the influence of vitamins and other substances when added to a deficient basal medium containing egg-white. This approach offers real promise of achieving bacteria-free cultivation and thus truly controlled experimental conditions. The qualitative influence of various chemical agents has been reported frequently, but conflicting results and uncontrolled conditions leave much to be desired.

Analysis of the growth requirements of microorganisms necessarily involves the reactions of populations, although the present authors have been unable to discover any studies of *E. histolytica* in which the course of population growth has been followed. Indications of cyclical trends have been noted by various investigators. For example, Craig (14) summarized many years of outstanding experience with *E. histolytica* by stating that "the number of amebae present

<sup>1</sup> This investigation was supported by a grant from the Abbott Fund of Northwestern University.

in the culture varies considerably at daily intervals and there appears to be some indication of a more or less regular cycle in the growth and multiplication of the organisms so far as numbers are concerned" (p. 241). It was decided to attempt to trace the growth of populations of *E. histolytica*, to permit comparison with other microorganisms and to express amoebic growth as a function of other variables operating in the culture medium.

#### MATERIALS AND METHODS

The NRS strain of *E. histolytica* was used in these experiments.<sup>2</sup> To obtain a more uniform stock a clone was isolated in 1943 and has been maintained since then. The associated bacterial flora includes three gram-negative bacilli, one of which is a strain of *Escherichia coli*, while the others seem to be enteric variants which do not match any species in Bergey (15).<sup>3</sup> This amoeba-bacteria combination has been used in all the experiments reported here, although comparative runs made with less homogeneous stocks of amoebae have given similar results.

Growth was studied in the media of Cleveland and Collier (6) (liver-infusion agar plus serum-saline overlay); Dobell and Laidlaw (2) (inspissated whole-egg slants plus serum-saline overlay); and Balamuth and Sandza (4) (buffered, aqueous egg-yolk infusion). Four modifications of the last were compared: stock infusion (four yolks in 250 ml. medium); stock infusion containing 0.5% Wilson liver concentrate powder 1-20; stock infusion containing 4% horse serum; dilute infusion (two yolks in 250 ml. infusion). The above media were carefully chosen in order to compare the growth-promoting qualities of liver- and egg-media in the diphasic and monophasic condition. Aqueous egg-yolk infusion seemed to offer special possibilities because it contained less complex nutrients than the other two media and did not stimulate bacterial growth as strongly.

Purified rice starch in 20-mgm. amounts was added as supplement to all cultures, and the amoebae were conditioned in a given medium for several weeks before each experimental run.

Large Pyrex test tubes (20 x 2.5 cm.) were used as culture vessels, with 40 ml. medium per tube. Each experimental tube received a one-ml. inoculum from a thoroughly mixed 48-hour stock culture. All experiments were conducted at least twice, and in each run duplicate tubes were used together with controls to test the effect of stirring and removing medium. It was necessary to extract media for counting purposes from well-stirred tubes, owing to the clumping of amoebae in the butt.

The counting techniques were adapted from standard methods. The bacterial population was counted by the decimal-dilution-plate method (16), employing one-ml. samples of medium and through trial runs adjusting the terminal dilutions to yield approximately 30-300 colonies in nutrient agar plates. Plates

<sup>2</sup> Obtained in 1943 through the courtesy of the Army Medical School.

<sup>3</sup> Work is in progress in this laboratory analyzing the relative influence of this flora on the growth of *E. histolytica*.



were prepared for each of the last two dilutions, to obtain a general check, and were incubated for 48 hours before counting. The amoebae were counted on Spencer bright-line haemocytometers by Paulson's method (17), using the average of six counting squares for each point on the growth curve. Determinations of pH were made electrometrically with a Coleman pH meter (model 3D), using for each test 0.4 ml. medium in a micro-chamber glass electrode.

One special series investigated the effect of using preconditioned stock egg-yolk infusion. Twenty-four-hour cultures of the bacterial flora alone in this medium were subdivided into three fractions: heat-treated (autoclaving for 20 minutes at 15 pounds pressure), Seitz-filtered, and untreated portions. The usual inocula of bacteria and amoebae were then added to each tube and growth was traced. At the time of inoculation, therefore, the untreated fraction con-

TABLE I

*Summary of data\* showing growth of populations of Entamoeba histolytica and the associated bacterial flora in several culture media*

CULTURE MEDIUM	INOCULUM (NO. PER CU. MM.)		YIELD (NO. PER CU. MM.)			
	Bacteria ( $\times 10^3$ )	Amoebae	Bacteria ( $\times 10^3$ )	Age	Amoebae	Age
				<i>hrs</i>		<i>hrs.</i>
Balamuth and Sandza.....	7.9	5.0	304	80	397	73
Balamuth and Sandza + horse serum.	9.6	6.9	339	61	359	59
Balamuth and Sandza + liver ex- tract.....	14.9	12.3	610	53	486	52
Dobell and Laidlaw.....	16.7	3.7	568	72	403	78
Cleveland and Collier.....	17.5	12.3	510	94	634	61

\* The figures in each case represent the mean values of at least four individual experiments.

tained a 24-hour-old population of bacteria, while the other two tubes contained no viable bacteria.

## RESULTS

1. *Growth in the standard media.* The data in the present experiments offer several aspects for study. The comparative yields of bacteria and amoebae in the different culture media<sup>4</sup> may be observed in table 1, while the course of growth lends itself most readily to graphical representation (figs. 1-6).

Table 1 indicates that, in general, higher yields of both bacteria and amoebae

<sup>4</sup> Counting techniques using small samples of populations inevitably introduce variability in results. In the present experiments this problem has been recognized by running duplicate tubes, repeating experiments, making several counts at each time interval, and plotting the data to obtain a general check on the *trends* of growth. No specific claims are made as to the absolute values obtained, but the conclusions reached seem justifiable on the basis of the consistent differences in values at different ages and between different culture media.

were produced in the richer, more complex media. The table also discloses a higher *average* inoculum in these media, although no specific attempt was made to study the influence of size of inoculum upon yield. In examining specific experiments, however, no regular correlation could be found. Thus

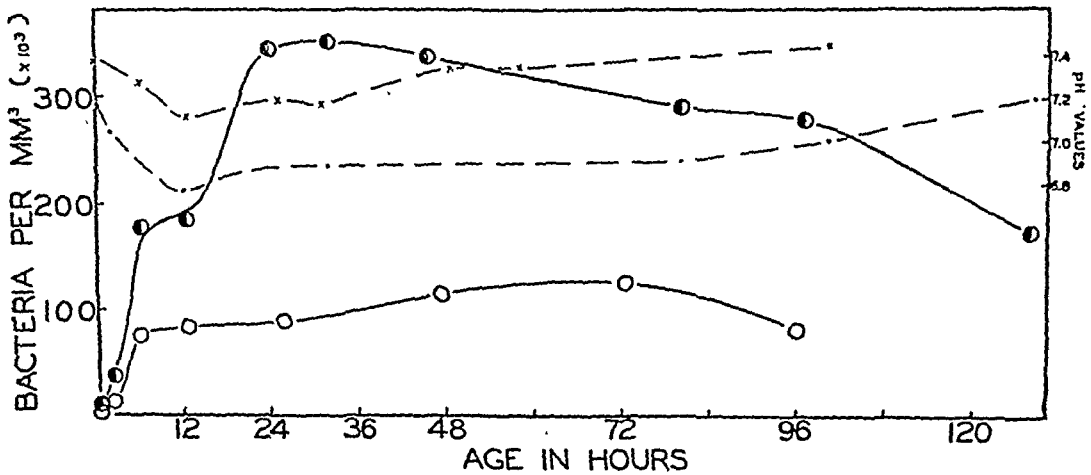


FIG. 1. The course of bacterial growth (● stock egg-yolk infusion; ○ dilute egg-yolk infusion) and changes in pH (· stock infusion; × dilute infusion) in the culture medium of Balamuth and Sandza.

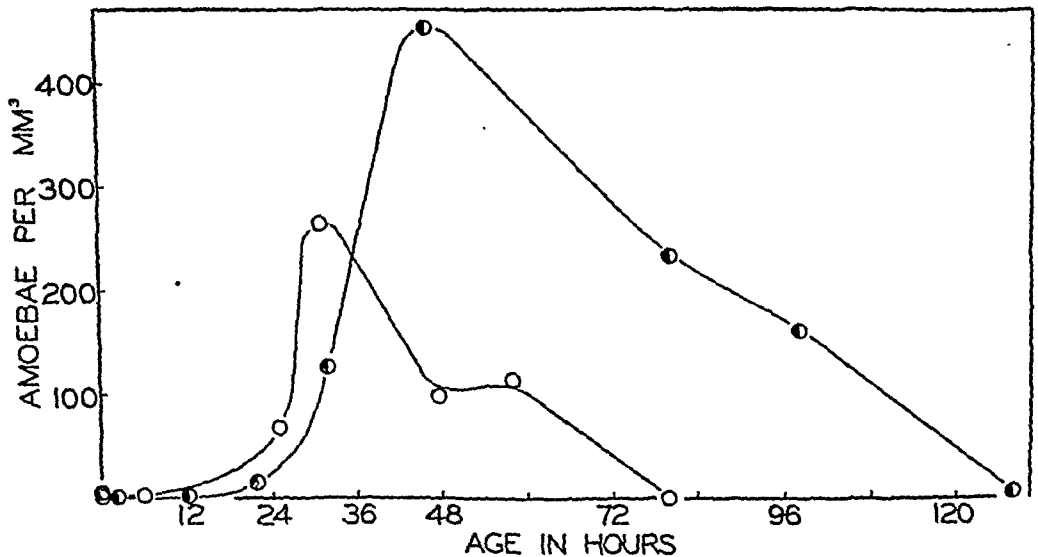


FIG. 2. The course of growth of populations of *E. histolytica* in the culture medium of Balamuth and Sandza: ● stock egg-yolk infusion; ○ dilute infusion.

in one run involving Cleveland and Collier's medium, a relatively small bacterial inoculum ( $7.4 \times 10^3$  per cu. mm.) produced a relatively large bacterial yield ( $606 \times 10^3$  per cu. mm.), compared to the average. A similar lack of correlation was evident in some cases of amoebic growth; for example, in a run involving

two cultures of Balamuth and Sandza's egg-yolk infusion plus 0.5% liver concentrate. A constant bacterial inoculum in both cultures of  $12 \times 10^3$  per cu. mm. and differing concentrations of amoebae (16 and 5 per cu. mm.) produced corresponding amoebic yields of 458 and 606 per cu. mm. In short, the individual yields seemed to be a closer function of the media themselves than of

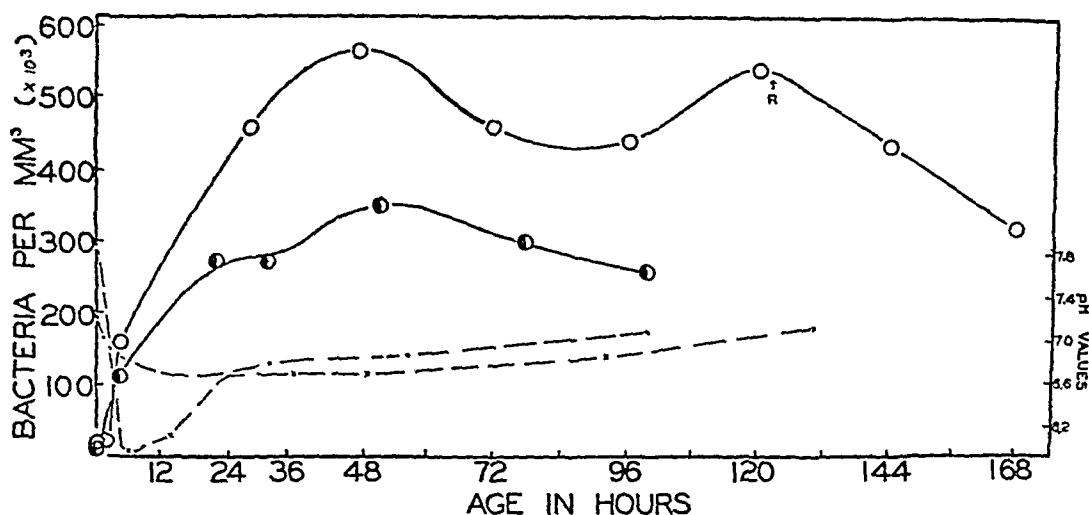


FIG. 3. The course of bacterial growth in the culture media of Balamuth and Sandza containing 4% horse serum (●), and of Dobell and Laidlaw (○); and changes in pH (· B. and S. plus serum; × D. and L.).

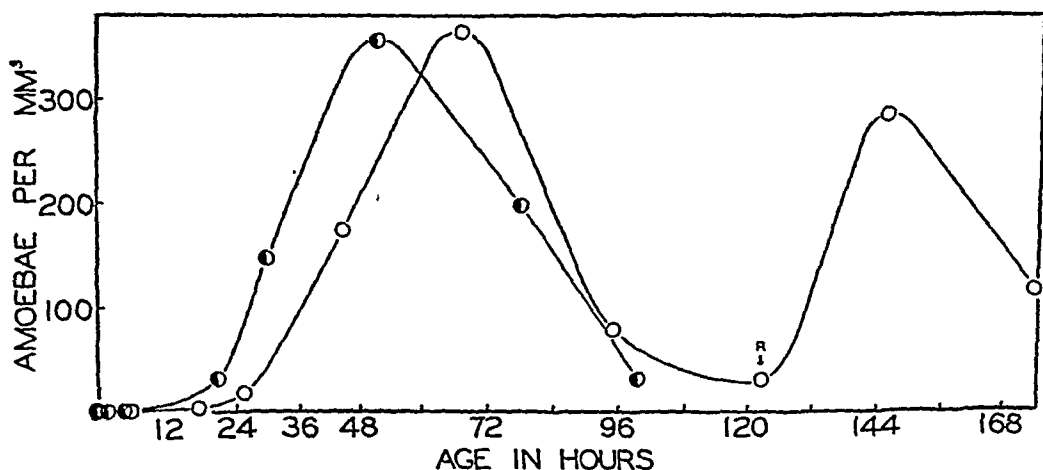


FIG. 4. The course of growth of populations of *E. histolytica* in the culture media of Balamuth and Sandza containing 4% horse serum (●) and of Dobell and Laidlaw (○).

the size of the initial population, as would be the case if the important limiting factor were in the growth-promoting capacity of the nutrients.

The course of growth in the several media was plotted on typical growth curves. Each curve describes a representative experiment, in contrast to the *average* values given in table 1. The curves for bacterial growth and pH changes have

been separated from amoebic growth for purposes of clarity, and the media have been grouped to permit comparison between standard media and modifications of Balamuth and Sandza's medium made up to simulate these in regard to certain nutrients.

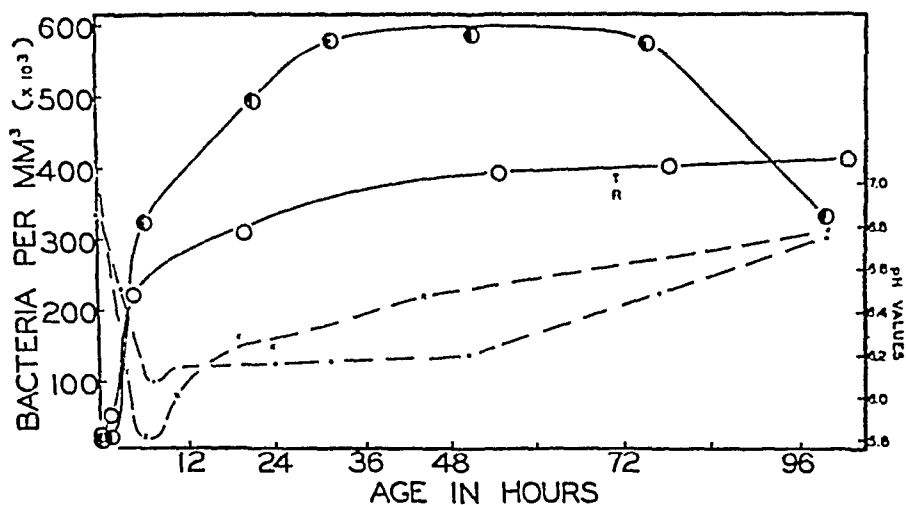


FIG. 5. The course of bacterial growth in the culture media of Balamuth and Sandza containing 0.5% Wilson liver concentrate (●); and of Cleveland and Collier (○); and changes in pH (· B. and S. plus liver; × C. and C.).

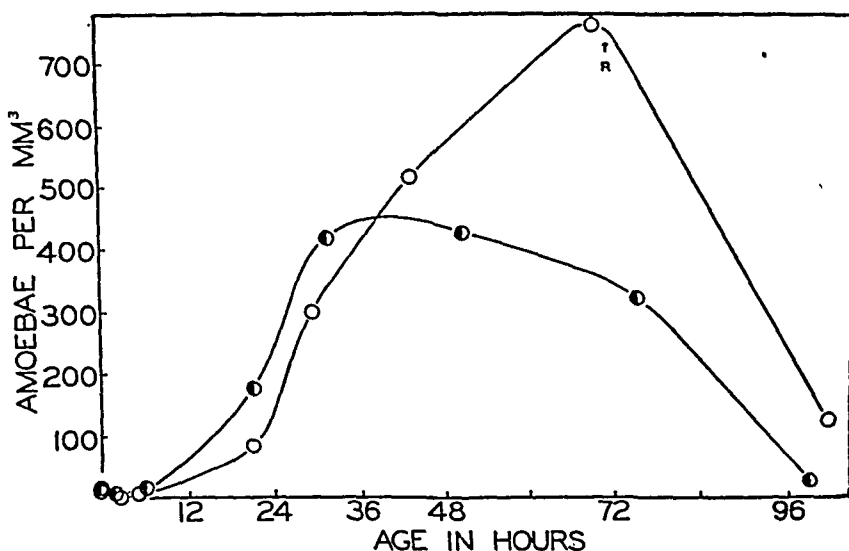


FIG. 6. The course of growth of populations of *E. histolytica* in the culture media of Balamuth and Sandza containing 0.5% Wilson liver concentrate (●) and of Cleveland and Collier (○).

The growth curves of the bacterial flora and amoebae typically assumed the classical bacterial pattern in each medium, exhibiting characteristic phases of lag, logarithmic growth, negative growth acceleration, maximum stationary growth, and accelerated death. It is striking that the lag phase was relatively

brief for the bacterial flora, while it was relatively prolonged for the amoebae. The logarithmic phase of amoebic growth began in all cases between 12-24 hours. It is clear, however, that at 24 hours more abundant growth was at-

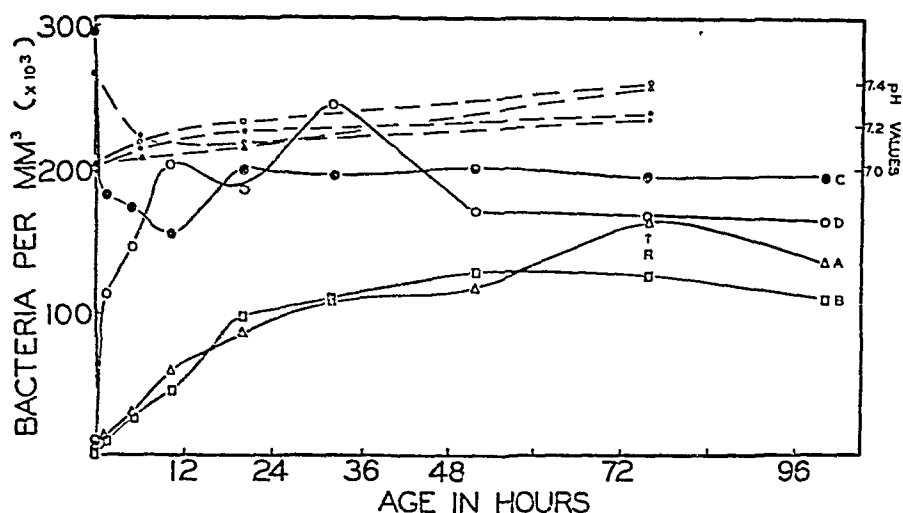


FIG. 7. The course of bacterial growth and changes in pH in fractions of the culture medium of Balamuth and Sandza preconditioned by growth of the bacterial flora and then treated in different ways before adding the usual inoculum: A (heat-killed flora); B (Seitz-filtered medium); C (untreated); D (control, as in fig. 1).

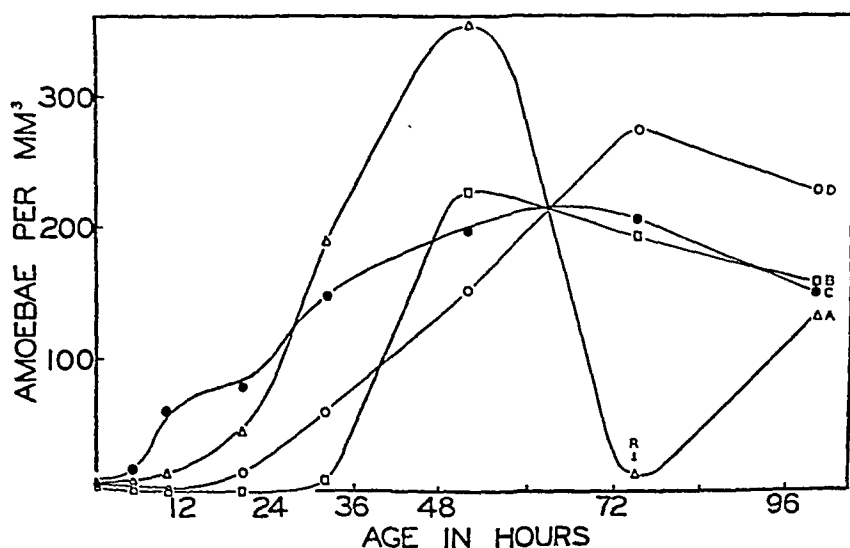


FIG. 8. The course of growth of populations of *E. histolytica* in the culture medium of Balamuth and Sandza preconditioned by growth of the bacterial flora and then treated in different ways before adding the usual inoculum: A (heat-killed flora); B (Seitz-filtered medium); C (untreated); D (control, as in fig. 2).

tained in those cultures showing greater initial bacterial growth (cf. figs. 1-2 with 5-6). Each class of bacteria-amoeba curves indicates this temporal dependence of beginning amoebic growth upon prior bacterial growth.

The onset of amoebic growth was not dependent upon a particular pH value in these experiments (figs. 1, 3, 5). There was a drop in pH during the first several hours in all cultures, which was correlated to rapid multiplication of bacteria. This was followed by a gradual shift toward the alkaline range. There was less fluctuation of pH in Balamuth and Sandza's medium, owing probably to the greater efficiency of its buffer system (11). In the general range tested in these experiments, pH did not seem to play an important role in limiting growth.

Qualitative observation of the amoebae shows that they uniformly underwent "shock reaction" at the outset, rounding up and eliminating some ingesta. During the first 6-12 hours, in fact, there was an actual decrease in number, and degenerating individuals could be seen. This was followed by marked activity on the part of the survivors and a growth rate of spectacular proportions, as evidenced by the steep slope of the growth curves. Although only living amoebae were counted (as judged by retention of a sharply refractile boundary of the body), there was definitely a higher percentage of active trophozoites in the egg-media than in the liver-media. There were evidences of encystment in the latter, together with many sluggish, vacuolated individuals even during periods of rapid growth.

Control tubes were set up with each series and were compared with experimental tubes only at 48 and 96 hours to test the influence of stirring and removing medium. The data indicate that there was consistently less bacterial and amoebic growth in the control tubes, despite the removal of medium from the experimental tubes. In one run in Balamuth and Sandza's medium, for example, the population of amoebae in the experimental tube at 48 hours was 452 per cu. mm. while the control tube contained 203 per cu. mm. The bacterial flora showed the same trend to a lesser degree. Similar data were gathered for each series. Stirring apparently had a positive influence on growth, and this factor warrants additional study.

The specific influence of rice starch upon amoebic growth was demonstrated in an interesting manner. It had been evident that the media became depleted of most of the rice starch as the cultural cycles waned; the influence of this constituent was tested, therefore, by adding 5-mgm. amounts of rice starch to some of the series in the phase of accelerated death. A marked, specific stimulation of amoebic growth could be noted in the egg-media while slight or no effect could be observed in the liver-media. Figs. 3 and 4 illustrate a positive effect in Dobell and Laidlaw's medium: the addition of rice starch at 122 hours had the pronounced effect of transforming rounded, vacuolated trophozoites into actively feeding individuals, even though the bacterial growth continued to decline. This was shown even more strikingly in a similar experiment involving two cultures in Balamuth and Sandza's stock infusion, the curves drawn in figs. 1 and 2 representing an untreated tube while the companion culture (not depicted) showed a similar trend until rice starch was added at 98 hours. In the following 24 hours the bacteria had continued to decline in both tubes (in the companion culture from  $303 \times 10^3$  per cu. mm. to  $145 \times 10^3$  per cu.

mm.), while the amoebae increased sharply in the companion tube from 124 per cu. mm. to 220 per cu. mm. The absence of this effect in liver-media is illustrated by Cleveland and Collier's medium in figs. 5 and 6. The addition of rice starch at 70 hours did not check the decline of the amoebae, while the bacterial population remained about the same. In no case did the late replenishment of rice starch give higher maxima than achieved in the earlier phases of growth, but the response does indicate that the basal constituents of egg-yolk media are not exhausted as rapidly as liver-media, and that rice starch has a specific effect upon amoebic growth.

2. *Growth in preconditioned media.* A group of experiments was designed to attempt to cast some light on the nature of the factors conditioning amoebic growth. One of these experiments is summarized in figs. 7 and 8, although two separate runs gave similar results. Balamuth and Sandza's medium was used because of its aqueous base and ease of handling.

Certain points deserve emphasis. Culture *D* served as a control, involving non-preconditioned medium as in the case of figs. 1 and 2, and showed a similar though slightly lower growth response. The curves for cultures *A* and *B* indicate that heating and filtration did not differ significantly from each other in affecting the potentialities of subsequent bacterial growth (fig. 7). In the case of the amoebae, however (fig. 8), both the untreated (*C*) and heat-treated (*A*) media exhibited shorter lag phases than the control (*D*), suggesting that the bacterial flora provided a heat-stable factor (or factors) stimulating the onset of amoebic growth. This influence was not directly dependent upon the continued presence of the bacteria, since the heat-treated medium (*A*) produced more amoebae and less bacteria than either cultures *C* or *D*. Maximal amoebic growth was reached earlier in all the preconditioned media than in the control. Seitz-filtration (curve *B*) apparently removed some factor (or factors) having specific influence on the amoebae, since the lag phase was definitely prolonged despite the fact that bacterial growth paralleled that of the heat-treated culture (*A*). This factor must be heat stable, and might well comprise nutrient products of bacterial metabolism. Finally, it might be noted that culture *A* at 75 hours received additional rice starch, owing to its depletion; this resulted as before in selective stimulation of amoebic growth.

#### DISCUSSION

To achieve the goal of bacteria-free cultivation of *E. histolytica* demands the analysis of all factors operating in the *in vitro* microcosm containing amoebae and their associated bacterial flora. The approach opened by the present work emphasizes some of the general cyclical changes which occur in amoebic cultures. The information thus obtained is simply a starting-point for more precise control of the milieu, including study of the relative contributions of members of the bacterial flora, the relation of size and growth phase of inoculum to size of yield, the influence of physicochemical factors (e.g., oxygen tension, substratum) and of specific nutrients—in short, progressive substitution of known for unknown factors. It is hoped that valuable insight may be gained by comparing

rates of response between experimental and control cultures, as in the above case involving preconditioned media.

The basic question raised by the present work concerns the lag phase in amoebic growth, and the effect on it of the bacterial flora. It is obvious that an inhibitory influence acts upon the amoebae during the first several hours of cultivation, following which time highly suitable conditions are introduced. Changes in pH have been eliminated as a primary influence (owing to lack of correlation in figs. 1, 3, and 5). The presence of excess free oxygen is definitely harmful to *E. histolytica*, as hanging-drop cultures demonstrate. This factor seems to be ruled out as primary in these experiments, however, since it was the same at the outset in the treated, preconditioned media and the stock egg-yolk infusion, although the lag phases were very different. Moreover, the aqueous media were boiled and cooled to 37°C. just before use to minimize the effect of free oxygen, and the presence of high concentrations of aerobic bacteria would of itself render this factor negligible (18).

From these considerations it seems probable that the bacteria must provide some of the responsible factors during the first few hours of cultivation. This view is supported not only by the use of preconditioned media, but also by the fact that there is a shorter lag in amoebic growth in those media in which early bacterial growth is greater (cf. figs. 1 and 2 with 5 and 6). It is especially upon the early phases of growth, therefore, that more attention must be focused.

The specific role of the bacterial flora is still undetermined, but two obvious possibilities exist: 1) that products of bacterial metabolism are used by the amoebae; 2) that the medium is adjusted through bacterial activity to a physicochemical range in which the amoebae can survive. The first possibility has not been explored. Chang (12) recently has offered evidence to show that the bacterial flora adjusts the oxidation-reduction potential of the medium to a highly reduced level, at which the amoebae thrive. In the absence of this reduced state the amoebae grow poorly and eventually die. This general condition is not unexpected in view of the anaerobic life of *E. histolytica* (10), but Chang's work opens a fruitful approach in correlating amoebic growth to the physicochemical state of the medium. The use of preconditioned media as in the present experiments may cast light on the relative importance of the adjustment of the medium and specific products of bacterial metabolism in promoting amoebic growth.

The stimulating effect on growth brought about by frequent agitation of the medium may have a simple explanation. The amoebae (and some bacteria) grow in local "nests" at the bottom of culture vessels. Stirring breaks up these clumps and scatters individuals over the substratum. In this way more effective use is probably made of the available bottom surface.

Counting bacteria offers more inherent difficulty than amoebae, owing to the tendency of some bacteria to grow in masses and of others to grow in short chains. The present data indicate, however, that it is possible to obtain a roughly quantitative expression of bacterial growth with good agreement at different times and by different counters. Several kinds of agar were compared



but Bacto-nutrient agar proved as suitable as any. Since the last two dilutions were plated in each case an independent check was obtained, and some series were discarded when it became evident that experimental errors had occurred.

The buffered, aqueous culture medium of Balamuth and Sandza offers several advantages over certain other standard media. It is practically colorless, it can be autoclaved and handled easily, and its nutrient base can be modified within fairly wide limits and still support growth. The present experiments indicate again (cf. 19) the dispensability of liver- and serum-supplements in the cultivation of *E. histolytica*. On every count this medium seems preferable to Dobell and Laidlaw's medium for routine cultivation. Cleveland and Collier's medium produced greater total yields in advanced stages of cultivation, but addition of liver supplement to Balamuth and Sandza's medium gave higher yields through the first 24-36 hours of growth (for a typical case see fig. 6). Cleveland and Collier's medium is difficult to handle because of extensive gas formation and the tendency of amoebae to clump beneath the easily loosened agar slant. Repeated observations showed greater uniformity of active trophic amoebae in egg-media than in liver-media, and this has led the authors to prefer the former in maintaining stock cultures. In the search for factors influencing amoebic growth, it is believed that aqueous egg-yolk infusion offers inviting possibilities.

#### SUMMARY

Growth of *E. histolytica* was traced in the culture media of Cleveland and Collier, Dobell and Laidlaw, and Balamuth and Sandza. The growth curves of amoebae and bacteria followed the classical bacterial pattern, although higher yields and greater change in pH occurred in the richer media. The lag phase of amoebic growth was relatively prolonged in comparison to bacterial growth. The data suggest a conditioning effect of the bacterial flora on the medium rendering it more suitable for amoebic growth.

Analysis by means of growth curves permitted the demonstration that rice starch had a specific growth-promoting influence on amoebae in late stages of cultivation, even though bacterial growth continued to decline.

Experiments showed that the lag phase of amoebic growth was shortened in media preconditioned by growth of bacteria alone. The data in general suggest the stimulation of amoebic growth by a heat-stable factor (or factors) produced by bacterial metabolism.

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# CULTIVATION OF ENDAMOEBA HISTOLYTICA IN ARTIFICIAL MEDIA FROM CYSTS IN DRINKING WATER SUBJECTED TO CHLORINATION\*

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The purification of drinking water for troops in the field is of paramount importance and a problem of continuing investigation. It is generally agreed that chlorination will destroy pathogenic bacteria but no agreement has been reached as to practical methods for the killing of the cysts of *Endamoeba histolytica*. Recently Chang (1) has indicated that, under properly controlled conditions chlorine may be used to kill these cysts along with the bacteria. He has stated that, at 18°C and a cyst density of 30-62 per ml., a concentration of chlorine of 2 parts per million will suffice provided the pH of the water is kept below 7.4 and organic matter in the water is low.

In studying his report it was noted that he used cysts derived entirely from amoebae that had been carried in the laboratory on artificial media for a considerable length of time. Since it is obvious that contamination of water will be by cysts derived from man or possibly other animals, and since these may well be more resistant than cultured cysts, it was decided to extend the work using cysts secured from human carriers. If these cysts were also uniformly killed then it would be possible under proper control to use chlorine for rendering water safe to drink in the field.

Chang has outlined carefully the conditions under which chlorine may be expected to kill the cysts in his Chart 1. At a pH of 7, temperature 18°C., cyst density of 30-62 per cubic centimeter, and total organic nitrogen of less than 0.2 part per million, the following relations prevailed between contact periods and lethal levels of residual chlorine: for 15 minutes contact, 4.0 parts per million of residual chlorine was cysticidal; for 30 minutes, 3.0 p.p.m.; for 60 minutes 2.0 p.p.m.; for 120 minutes, 1.0 p.p.m. These conditions were reproduced as closely as possible in the experiments here reported. Chang also reported that the oxidation-reduction potential of chlorinated water as expressed in millivolts was a more accurate measure of cysticidal efficiency. No equipment was available at this overseas station for taking such measurements.

## MATERIALS

The water used in the experiments was taken from a tap in the laboratory. It came from a 60 foot tube well from which it was pumped into a tank and distributed by pipe to the camp. It was ordinarily clear, with a pH of 6.4 to 6.6.

\* This investigation was carried out at the Ninth Medical Service Detachment (Laboratory), near Chabua, Assam, whose commanding officer, Lt. Col. H. A. VanAuken, authorized the project.

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The cysts of *Endamoeba histolytica* were collected from human feces. About 50 grams of feces heavily charged with cysts were mixed in several hundred cubic centimeters of distilled water and the mixture poured through a fine screen to remove coarse particles. The filtrate was diluted to 1100 cc. in a graduated cylinder, shaken, and left to stand overnight. The supernatant was siphoned off, the sediment diluted with distilled water and divided into 50 cc. centrifuge tubes. The sediment was washed repeatedly with centrifugalization, usually 7 or 8 times, until the supernatant was clear. After this treatment practically no mucus or odor of feces remained in the finely divided residue. The latter was diluted with about 300 cc. of distilled water, counts made to determine the cyst density, and the cyst suspension was stored in the refrigerator until used. Cysts of the "large race" of *Endamoeba histolytica* from 3 different soldiers were employed in the experiments. Several attempts were made to experiment with a "small race," but it could not be grown in culture.

The Chang (2) (1942) modification of the Cleveland medium and our own modification of the Boeck medium were used for culturing *Endamoeba histolytica* from cysts. The slants for the former were made according to the directions on the label of the bottles of Bacto Entamoeba Medium (dehydrated). The overlay was made according to Chang with slight modification. It consisted of 1 part fresh sterile inactivated human serum and 10 parts of the following solution:  $\text{Na}_2\text{HPO}_4$ , 4.447 gm.;  $\text{KH}_2\text{PO}_4$ , 0.269 gm.;  $\text{NaCl}$ , 8.0 gm.; distilled water, 1000 cc. The solution was autoclaved before mixing with the serum and the mixture was then passed through a Seitz filter to insure sterility. The slants for the modified Boeck medium were made as follows: 60 cc. dried whole egg powder (Army issue) were thoroughly mixed with 35 cc. of Locke's solution and 135 cc. of distilled water with the aid of glass beads. Four to five cubic centimeters of this mixture were placed in each tube and the tubes plugged. The slanted tubes were buried in saw dust and autoclaved for 10 minutes in flowing steam, followed by autoclaving for thirty minutes at 15 lbs. After cooling the tubes were removed from the sawdust and the overlay, consisting of Locke's solution, 8 parts, and sterile human serum, 1 part, added. Both types of medium were incubated 48 hours for the sterility test. Just before use to each medium was added a small amount of sterile rice flour. We preferred the Chang-Cleveland medium, for at times a breakdown was noted in the egg slants.

#### PROCEDURE

The steps in the experimental procedure adopted after a number of preliminary trials were as follows:

1. 1200 cc. of tap water in a 2 liter Erlenmeyer flask placed in a water bath were brought to 18°C.
2. 200 cc. of the water were withdrawn from the flask and mixed with sufficient cyst suspension so that when returned to the flask the final concentration would equal 25 to 40 cysts per cubic centimeter.
3. To the 1000 cc. of water remaining in the 2 liter flask sufficient 0.25 per cent calcium hypochlorite solution was added to bring the residual chlorine to the

## RESULTS

Table 1 records 22 experiments in which it was possible to culture *E. histolytica* from cysts exposed to varying concentrations of chlorine for different periods of

TABLE 1

*Chlorine concentration and total N in parts per million during contact periods at ends of which positive Endamoeba histolytica cultures were obtained from treated waters adjusted to 18°C. and pH 7.0*

EXPERIMENT	HOST SOURCE OF CYSTS	CONTACT PERIOD (MINUTES)	RESIDUAL CHLORINE AFTER 10 MIN. CONTACT*	RESIDUAL CHLORINE AT END OF CONTACT PERIOD	TOTAL N
A	Taylor	20	Not taken but not less than 2.0	2.0	0.40
		30		2.0	0.40
		60		1.5	0.40
		120		0.5	0.40
B	Taylor	30	2.0	2.0	0.32
		60	2.0	1.8	0.32
		120	2.0	1.0	0.32
C	Picotte	15	2.0	2.0	0.16
		30	2.0	2.0	0.16
		60	2.0	2.0	0.16
		120	2.0	1.5	0.16
D	Picotte	30	2.0	2.0	0.16
		60	2.0	1.5	0.16
		120	2.0	1.0	0.16
E	Picotte	30	2.0	2.0	0.16
		60	2.0	2.0	
F	Picotte	60	2.0	1.5	0.20
G	Picotte	30	10.0+	8.0	0.20
H	Picotte	30	5.0	3.5	0.28
I	Picotte	30	5.0	3.0	0.28
J	Picotte	30	8.0	6.5	0.28
K	Wilson	60	10.0+	10.0	0.12

\* Values of "2" were probably actually higher, because the testing set would not read higher than 2 p.p.m.

time. There were also many other experiments in which cultures were not obtained.

It will be noted that most of the tests were carried out with a concentration of 2 parts per million of residual chlorine, tested 10 minutes after adding the cysts.

This concentration was used for the most part because it is not too great a departure from standard Army practice which requires 1 to 2 p.p.m. in the Lyster bag 10 minutes after mixing hypochlorite and water and because of the results of Chang reported above. The total nitrogen varied from 0.12 to 0.40 p.p.m., a range not excessive in comparison with Chang's conditions of 0.1-0.2 p.p.m.

After the failure to destroy the cysts of *E. histolytica* at the lower chlorine levels it was decided to try greater concentrations. Table 1 shows that viable cysts remained in the water after exposure to 5, 8 and 10.0 parts per million of chlorine with total nitrogen of 0.2 to 0.28 p.p.m. for periods of 30 min., and in one case after at least 10 p.p.m., for 60 minutes. In this instance the total nitrogen was only 0.12, the cysts having been passed through a sand filter an inch thick before being washed with the centrifuge. The sand filter was very effective in excluding much particulate matter other than cysts.

#### DISCUSSION

It is evident from the results presented that destruction of the cysts of *E. histolytica* by hypochlorite chlorination with the addition of adjustment of the pH to approximate neutrality, cannot be accomplished with any certainty. The conditions maintained in the experiments were close to those recommended by Chang. Our work differed in three respects: (1) The cysts were obtained directly from human hosts while his came from cultures that had been carried for a considerable time on artificial media; (2) our cysts were from 3 different human hosts; (3) a different cultivation procedure was used.

The reasons for using cysts derived from the human host are given above. In culturing the exposed cysts our practice was first to transfer the sediment to starch-free tubes.

At the end of 24 hours transfers were made to the same medium to which a small amount of rice starch had been added. If these subcultures were positive at the end of 48 hours the experiment was recorded as positive. If no growth was observed the sediment in each tube was again subcultured. If motile trophozoites were found in any of these cultures the result was considered positive. Chang followed the routine procedure of inoculating one tube of medium and examining it from the fourth to the seventh day, although he did make a few subcultures. He states that in no case did a negative culture turn out positive on subculture. In a number of instances our second subculture was positive when the first had not been.

It was our experience that it was much easier to cultivate trophozoites from cysts in 2 p.p.m. of chlorine for 15 to 20 minutes than it was following longer exposure to this or higher concentrations. There were many failures to obtain positive cultures, but these are not recorded here because, as is well known, there are many factors other than chlorination which govern success or failure in culturing from cysts of *E. histolytica*. In addition, the incubators available were very difficult to regulate, and it would have been impossible to differentiate between cultures lost through chilling or overheating and failure to grow owing to chlorination. Therefore not much significance could be attached to negative

results. It is our belief, however, that chlorine either kills a great many of the cysts or induces in them a lag period from which some of them are able to recover in due time in culture media. It is conceivable that both phenomena occur. Whatever the explanation, the practical significance is that since chlorine does not kill all of the cysts, it is unsafe to depend upon it for rendering drinking water potable so far as possible contamination with *E. histolytica* is concerned.

#### SUMMARY AND CONCLUSIONS

1. Experiments are reported on the cultivation of the cysts of *Endamoeba histolytica* following controlled exposure to varying strengths of residual chlorine in water.

2. In a number of instances the cysts remained viable after such treatment.

3. Chlorination of drinking water in practical amounts cannot be depended upon to destroy all of the cysts of *E. histolytica*.

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# STUDIES OF THE DISSEMINATION OF CYSTS AND OVA OF HUMAN INTESTINAL PARASITES BY FLIES IN VARIOUS LOCALITIES ON GUAM

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The prevalence of intestinal parasites among the natives of Guam has been amply demonstrated in studies undertaken by members of the Parasitology Laboratory of this Unit under the direction of Lt. Commander N. R. Stoll, H(S), USNR (1). Among the possible modes of spread of intestinal parasites on the island, dissemination of cysts and ova by flies was considered particularly worthy of investigation, because of the fact that numerous flies are found in certain of the native villages. *Chrysomya megacephala*, a species much larger than the house fly, is the most conspicuous one. Its body length measures up to 15 millimeters. It is well known that flies may be involved in the transmission of parasitic forms. Herms (2) credits Nicolle with having done the most extensive and careful work on the dispersal of eggs of parasitic worms by the house fly. Nicolle noted that while ova could be conveyed from excrement to food either on the surface of the house fly's body or in the intestines, the latter mode was practical only when the diameter of the egg was under 0.05 mm. He further found that eggs with a diameter of up to 0.09 mm. may be conveyed on the external surface; however, these eggs adhere to the body of the fly for only a short time, while those harbored in the intestines may remain there for two days or longer. Herms gives an extensive list of helminths transmissible by flies. With regard to intestinal protozoa he states that Roubaud found that cysts of *Endamoeba coli*, *Endamoeba histolytica* and *Giardia lamblia* passed through the intestine of the fly uninjured, and that free amoebae (both *coli* and *histolytica*) when fed to flies were found dead in the fly's intestine in less than an hour; also that Root found motile *Chilomastix mesnili* in a fly's feces seven minutes after it had fed on a stool containing them. Craig and Faust (3) have summarized investigations of the role of flies in the spread of amebiasis: "It has been shown by several investigators (Thomson and Thomson, 1916; Wenyon and O'Connor, 1917; Roubaud, 1918; and Root, 1921), all of whom used the eosin-staining viability criterion, that the cysts may be found in a viable condition in the droppings of flies for as long as 48 hours after those insects have fed upon contaminated feces. Pipkin (1942), using culture technics, found that filth flies of five common species could retain these cysts for 86 to 258 minutes and later deposit them in a viable state. Frye and Meleney (1932) found cysts of the amoeba in the intestine of flies caught in 3 of 12 houses where individuals infected with the parasite resided. Both trophozoites and cysts of *Endamoeba histolytica* may be found in the vomitus of flies. Pipkin (1942) states

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that trophozoites were cultivable from the vomitus after periods of between 28 and 64 minutes."

#### METHODS

Lt. Commander G. E. Bohart, II(S), USNR, and other members of the staff of the Entomology Laboratory of this Unit provided us with valuable information

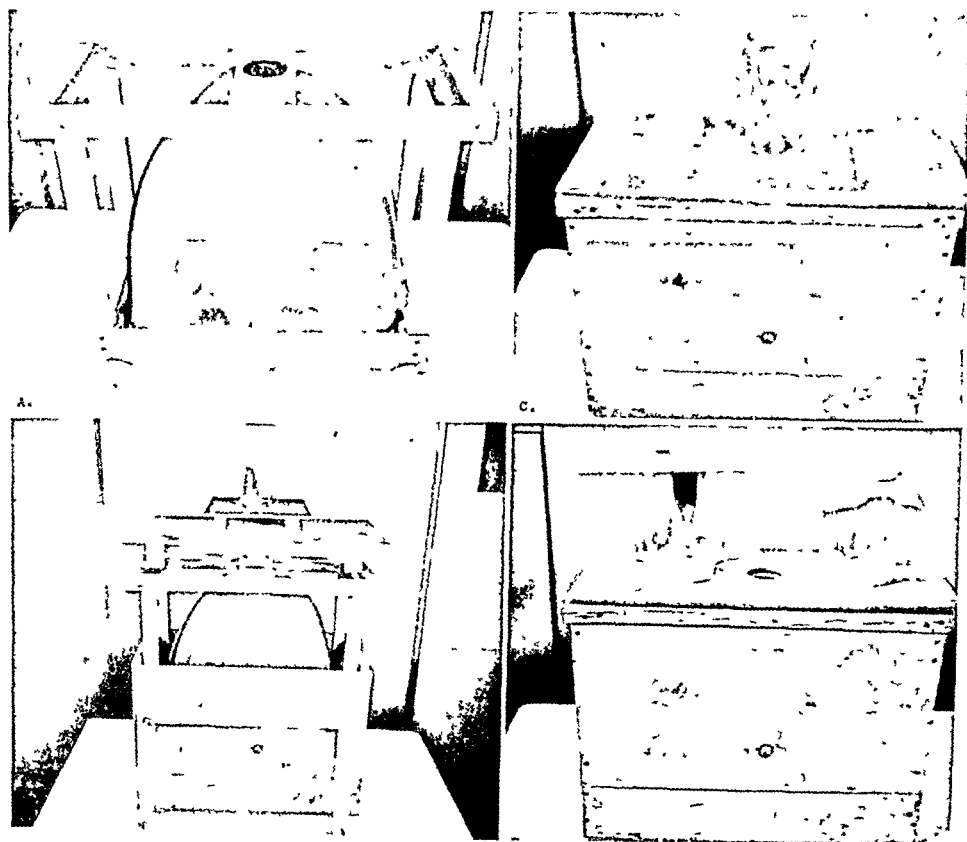


FIG. 1. A. Trap, with bait cup, for catching flies. B. Trap (off its stand), box, cover, cone, chimney, gauze disc and Petri dish, showing arrangement for removing live flies. C. The same, completely assembled. D. One chimney shown inverted, with cone ready to be removed. Another chimney, with muslin cover.

and assistance. Technics of catching flies alive, in the open, have been modified somewhat to meet our needs.

The traps themselves are constructed of wood and wire screening along conventional lines, as indicated in figure 1. When in use, the trap rests above the ground on a support and over the container holding the bait. The two inch hole at the top is plugged with a rubber stopper. Catches are generally made over a two to six hour period. The bait usually used is fresh, normal feces, protected by gauze from contact with the flies. In and near dwellings, stale fish is used.

In emptying the trap, the rubber stopper is removed and a plywood cover with a central hole of the same size is placed on top in such a manner that the hole in the trap is temporarily covered. Then a small, square, plywood board, also with a hole of the same dimensions, is placed on top; this board supports a cone of wire screening with a small aperture at the

apex. Over this cone a lantern chimney is inverted. A Petri dish containing a disc of absorbent gauze has previously been taped to the base of the chimney. When all has been assembled, the plywood cover is moved so that all holes are aligned and the assembly is lowered into the box. Since all light is thereby excluded from the trap, except that which enters through the hole in the top, the flies seek to escape by this route. Usually the trap becomes empty in two or three minutes. Once in the chimney, the flies are prevented by the cone from retreating. At this juncture, cone and chimney are inverted together so that the cone is uppermost. The latter is vigorously tapped to dislodge flies that remain on the outer surface of the cone, then quickly flipped off and replaced by a cover of unbleached muslin, subsequently fixed in place by a rubber band. A small hole in the muslin, closed by a cotton plug, permits the pipetting of sufficient 0.9 per cent salt solution onto the gauze to keep it damp while the flies ingest the moisture and deposit regurgitated liquids and fecal specks on the gauze.

After a period of time, generally from one to four hours, when the gauze appears to be well spotted, a few drops of chloroform are put on the muslin cover. The asphyxiated flies are removed, identified and counted. The moist gauze is thoroughly washed in 0.9 per cent salt solution. The fluid is then centrifuged at 2000 revolutions per minute, the supernatant is discarded, and a portion of the sediment is examined microscopically. Another portion is mixed with a little serum on a glass slide, fixed in Schaudinn's fluid and stained with Heidenhain's hematoxylin stain. The residue of the sediment is cultured on slants of Endamoeba medium (Difco), to which has been added fluid consisting of five parts of Locke's solution and one part of inactivated human serum, plus a two millimeter loopful or rice starch in the form of flour fine enough to pass through an 80 mesh screen.

The traps are not adapted to indoor use. The dejecta from flies in the kitchens of native homes are obtained in the following manner.

A gauze disc, placed in an open Petri dish, is soaked in a small amount of normal blood serum and a minnow's tail is placed in the center. The odor of the fish attracts the flies which ingest the serum and speck the gauze. After from one to four hours, the gauze is removed and handled according to the prescription in the paragraph above.

Individual fly specks have been examined in the following manner.

Microscope slides freshly covered by a layer of serum are placed near a latrine in a native yard. When a slide is specked by a fly the former is placed at once in Schaudinn's fluid. It is subsequently stained and examined microscopically.

In addition to the examination of fly dejecta to determine the presence of cysts and ova of medical importance in general, a special study has been made of the rôle of flies in the epidemiology of hookworm infection. The objective was to reproduce as closely as possible the actual conditions found in native yards, in some of which food is prepared on outdoor tables and scraps are allowed to accumulate on the ground where they attract swarms of flies. The method adopted is as follows.

Metal cans, size No. 10, with small perforations in the bottom, are filled with loosely packed, coarse earth and are sterilized by autoclaving. The earth is then saturated with rainwater. A morsel of frozen fish that has been allowed to thaw and to decompose slightly in a covered container in the laboratory is placed on top of the earth. The cans are protected by placing them in a cage of chicken wire, which is set in a shady spot in a native yard. The cans are left for a period of from five to seven days, after which time they are brought back to the laboratory. The surface of the earth is examined for the pres-

ence of larvae. The upper one to two inches of earth are then removed and examined by the Baermann technic.

All of the observations reported here were made between October 1, 1945 and March 1, 1946. Most of the studies were undertaken in the Village of Dededo. This village, in the north central part of Guam, was built in a new location after the reoccupation of the island by American forces. In this paper it is designated as Area A. A part of the old Village of Dededo remains; it is located near the ocean. It is designated as Area B. Except in the driest weather the ground in this area remains quite moist. As a corollary to the studies in Dededo an additional study was made at a single location in Agana, near a shell-torn building housing a restaurant. This locality will be called Area C. Merizo is an old village near the southern tip of Guam. Flies were caught in two premises in this village, to be referred to as Area D; one of the places was on slightly elevated ground (D-1), while the other was in a damp spot, being located near the bank of a stream and close to the ocean (D-2).

#### RESULTS

*Endamoeba histolytica*. Particular attention was paid to the dissemination of cysts of *Endamoeba histolytica*, being the incitant of one of the more important of the protozoan diseases of man. Five different areas were studied in Dededo Village, designated as Areas A-1, -2, -3, -4, and -5, all near latrines serving from one to four homes (see table 1). These areas were chosen because analysis of the results of the survey to which reference has been made (1), indicated that the incidence of amebiasis was high (see table 2). In material obtained from flies trapped near the latrines in Areas A-1, -2, -3, and -4 cysts of *Endamoeba histolytica* were identified. Both 12 mu and 8 mu cysts were cultivated and repeatedly subcultured from material obtained from flies in Areas A-1 and A-3. Eight mu cysts only were found in the catch at Area A-4; the strain was maintained in serial cultures. Attempts to cultivate the 12 mu and 8 mu cysts found in material from Area A-2 were unsuccessful, but they were positively identified by morphological characteristics, including typical chromatoid bodies. In a native yard in the part of the old Village of Dededo that still remains (Area B), traps were set on two occasions. Eight mu cysts of *Endamoeba histolytica* were found on both occasions, and 12 mu cysts on one. The cysts were successfully cultivated and repeatedly subcultured. As a corollary to these studies at Dededo, an examination was made of material from flies trapped in the rear of a public eating place in the Village of Agana, at a point about 20 feet from the only latrine in the vicinity (Area C). In spite of the fact that a careful examination of material from over 400 flies was made, cysts of *Endamoeba histolytica* were not found; the only parasitic form seen was one hookworm ovum. This discovery was in striking contrast to our experience at Dededo. The incidence of intestinal parasitism among the citizenry of Agana has been found in the past to be high (4) and records of admissions to the Military Government (Civilian) Hospital indicate that the condition persists. Therefore the conclusion was drawn that a majority of the flies must have fed in places other than the latrine. This belief was borne out

by information given by the proprietor of the restaurant who assured us that the pit of the latrine was burned out daily with gasoline. At Merizo, in the rear of a

TABLE 1

*Human intestinal parasites found in the dejecta of flies caught near five latrines and a garbage drum in the village of Dededo*

AREA	LATRINE	TOTAL NO. OF DAYS OF TRAPPING	TOTAL NO. OF FLIES CAUGHT	PARASITES IDENTIFIED
(A-1)	serving lots no. 14-1, -2, -3, -4.	15	1359	<i>Endamoeba histolytica</i> (12 mu and 8 mu cysts) <i>Endamoeba coli</i> <i>Endolimax nana</i> <i>Iodamoeba butschlii</i> <i>Giardia lamblia</i> <i>Chilomastix mesnili</i> <i>Trichomonas hominis</i> Hookworm <i>Trichuris trichiura</i> <i>Ascaris lumbricoides</i>
(A-2)	serving lots no. 14-5, -6, -7, -8.	6	374	<i>Endamoeba histolytica</i> (12 mu and 8 mu cysts) <i>Endamoeba coli</i> <i>Endolimax nana</i> <i>Giardia lamblia</i> <i>Trichomonas hominis</i> Hookworm <i>Trichuris trichiura</i>
(A-3)	serving lots no. 14-9, -10, -11, -12.	1	411	<i>Endamoeba histolytica</i> (12 mu and 8 mu cysts) <i>Endamoeba coli</i> <i>Endolimax nana</i> <i>Giardia lamblia</i> Hookworm <i>Trichuris trichiura</i>
(A-4)	serving lots no. 17-1, -2.	1	19	<i>Endamoeba histolytica</i> (8 mu cysts)
(A-5)	serving lot no. 13-2.	2	114	Hookworm <i>Trichuris trichiura</i>
	GARBAGE DRUM			
(A-1)	serving lot no. 14-4.	1	186	Hookworm <i>Trichuris trichiura</i> <i>Ascaris lumbricoides</i>

house among whose inhabitants there had recently been a case of amebiasis, a small bush was found, about 10 feet from the kitchen porch, that appeared to be

a favorite resting place of flies. Material obtained from flies trapped at this spot (Area D-1), contained 8 mu cysts of *Endamoeba histolytica*. Flies appeared to be abundant near the latrine of another lot in Merizo (Area D-2). This latrine was in a damp spot, being located near the bank of a stream and close to the ocean. Material from flies caught on the first occasion failed to reveal cysts of *Endamoeba histolytica*. However, about two months later a trap was set in the same yard; on this occasion it was placed directly beside an outdoor table where food was being prepared. Eight mu cysts of *Endamoeba histolytica* were identified, cultured and successfully subcultured. All of the foregoing data were obtained in the examination of pooled fly specks that had been deposited on gauze moistened with 0.9 per cent salt solution.

TABLE 2

*An analysis of intestinal parasitism in persons living in the part of Dededo Village included in the present study, prepared from data obtained in an earlier parasitological survey. (1)*

AREA	LOT NO.	NUMBER OF INHABITANTS	NUMBER IN WHOSE STOOLS THESE PARASITES WERE FOUND:					
			<i>Endamoeba histolytica</i>	<i>Giardia lamblia</i>	Other protozoa	Hook-worm	<i>Trichuris trichiura</i>	<i>Ascaris lumbricoides</i>
(A-1)	14-2	11	2	5	10	11	11	1
(A-1)	14-4	14	7	6	9	14	14	1
(A-2)	14-6	5	4	1	4	5	5	2
(A-2)	14-7	11	3	0	5	11	11	7
(A-2)	14-8	6	1	0	1	6	6	0
(A-3)	14-9	4	0	0	1	4	4	1
(A-3)	14-10	11	2	1	5	11	11	1
(A-3)	14-12	12	4	4	10	10	11	2
(A-4)	17-1	10	3	3	1	5	10	0
(A-4)	17-2	6	4	2	0	5	6	1
(A-5)	13-2	9	2	4	4	7	8	0

The presence of *Endamoeba histolytica* in flies lighting on the kitchen table in a house near which studies had been previously made (Area B). was readily demonstrated by the special method previously described. Cysts of *Endamoeba histolytica* (12 mu) were identified, and the microorganisms were successfully cultured. A single unsuccessful attempt was made to repeat these findings in each of three other houses (Area A-1, D-1 and D-2); it seems quite certain that repeated attempts would yield positive results in areas where flies have been found to be infected, but the observation of Frye and Meleney (3) having been confirmed further work relative to the finding of infected flies indoors has not been undertaken.

During the early part of these studies, a great number of individual fly specks were examined by the method described employing glass microscope slides

coated with serum and placed in Area A-1. This laborious procedure was superseded by the technic involving the pooling of fly specks deposited on moist gauze. However, it has permitted a study of the characteristics of the specks stained with Heidenhain's hematoxylin stain, in one of which a considerable number of cysts of *Endamoeba histolytica* with typical chromatoid bodies was seen (figure 2 A). The cysts were found intermingled with fecal debris in the specks, strongly suggesting that they had passed through the fly's gut.

*Hookworm.* Hookworm disease constitutes more of a medical problem on Guam than infection with any of the other helminths. Both *Ancylostoma duodenale* and *Necator americanus* are prevalent in the native population. Hookworm ova were found in the same pooled fly specks as *Endamoeba histolytica*, in most instances, as indicated in table 1. The garbage drum noted in table 1 was located in Lot No. 14-4 about 50 feet from the latrine and 15 feet from the kitchen doorway. The likelihood that at least an appreciable number of flies caught here had fed in a latrine was shown by the large number of hookworm ova found in their dejecta. Ova of hookworm were identified on both of the occasions in which material was obtained from flies in Area B. The single parasitic form that was found near the restaurant in Agana (Area C) was a hookworm ovum. Material from a large batch of flies caught in Area D-1 was not found to contain any hookworm ova. This circumstance is believed to have been due to the fact that almost all of the flies in this instance were small—evidently too small to transport effectively helminthic ova. The matter will be discussed further in the section relating to the flies that were caught. In Area D-2, flies deposited large numbers of hookworm ova on the moist gauze, many of which were seen to be embryonated.

The results of the experiments devised to study the possible rôle of flies in the spread of hookworm infection indicate that they may be of significance in this respect, at least on Guam. Cans of earth prepared as described were placed on three occasions in the yard of the home in Merizo (Area D-2) where the flies had been found to harbor numerous hookworm ova of which many contained motile embryos. The location was a shady spot between the kitchen door and the latrine, habitually traversed by the natives, often with bare feet. After from five to seven days during which time there were several brief showers, the cans were examined under a dissecting microscope. The surface of the soil appeared moist. The fish had been devoured, and at the spots where the fish had been placed, scores of nematode larvae were seen whose rearing motions under the warmth of the microscope lamp were typical of those of filariform hookworm larvae. Concentration was effected by means of the Baermann technic. While various nematodes in different stages of free-living development were seen under the compound microscope, a large number of the observed forms were morphologically typical of rhabditiform and filariform hookworm larvae. The larvae shown in figure 2 were isolated from the earth in these studies.

*Other human intestinal parasites.* Cysts of *Giardia lamblia* and ova of *Trichuris trichiura* and *Ascaris lumbricoides* were frequently encountered, as shown in tables 1 and 3. An ovum of *Hymenolepis diminuta* was found only once, in

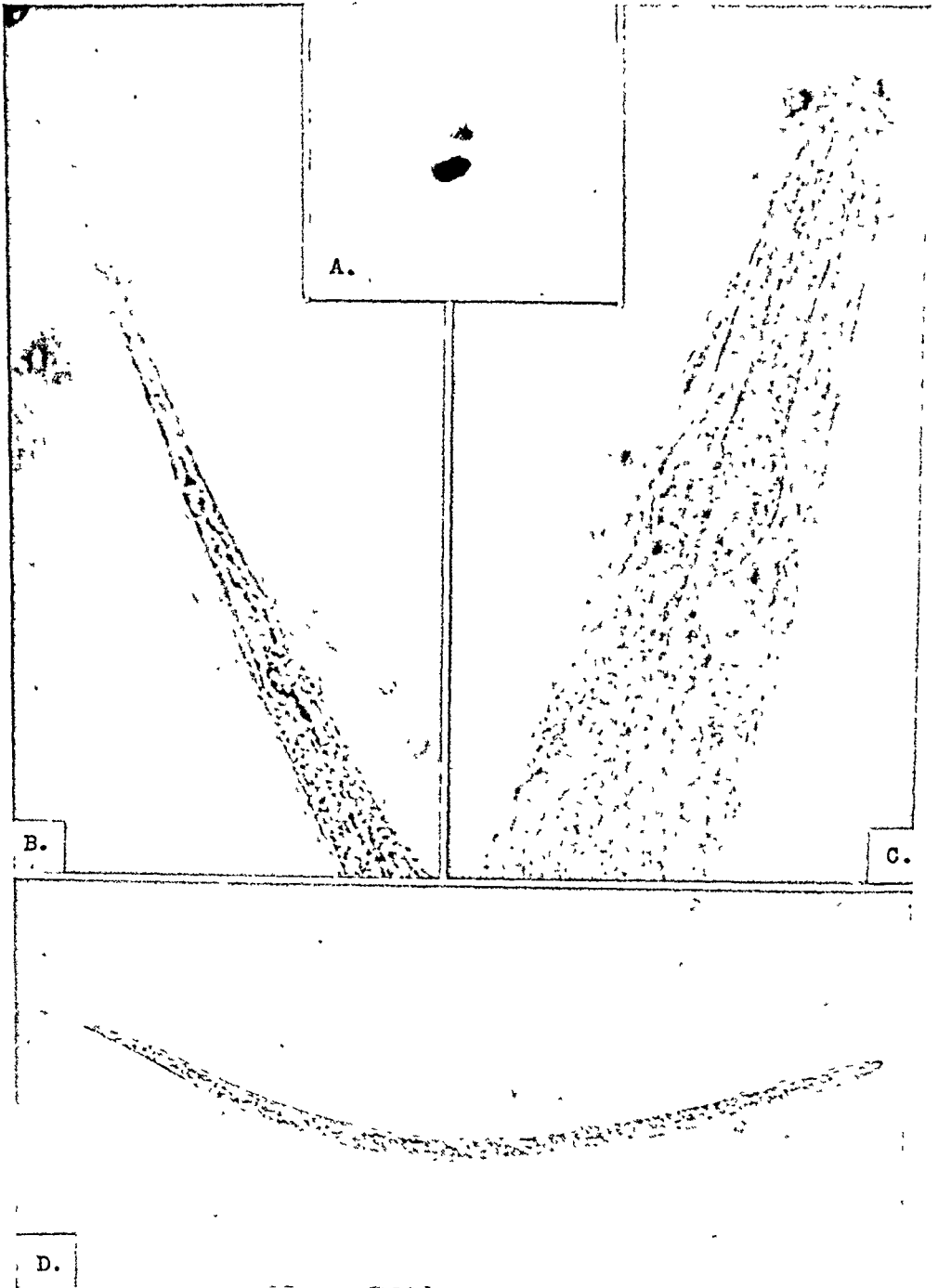


FIG. 2. A. Cyst of *Endamoeba histolytica* (12  $\mu$ ) in a fly speck. Larvae of hookworm recovered from earth contaminated by flies: B and C. Caudal and cephalic parts of a developing larva magnified to show certain internal structures. D. Filariform hook worm larva.

material from flies obtained in the second catch at Area B. Evidence of this parasite had not been previously encountered, and during the stool surveys

undertaken by members of the Parasitology Laboratory it was conspicuous by its absence. As one would expect, forms of parasites not found in human stools have been commonly observed along with those of medical interest in the studies of flies, and it seems quite likely that this ovum, although occasionally encountered in humans, was in this instance transferred by flies from the feces of a rat or some other animal. Certain species of protozoa that are frequently found in

TABLE 3

*Human intestinal parasites found in the dejecta of flies caught at locations in the old village of Dededo, in Agana, and in Merizo*

AREA	TOTAL NO. OF DAYS OF TRAPPING	TOTAL NO. OF FLIES CAUGHT	PARASITES IDENTIFIED
(B)	2	938	<i>Endamoeba histolytica</i> (12 mu and 8 mu cysts) <i>Endamoeba coli</i> <i>Giardia lamblia</i> Hookworm <i>Trichuris trichiura</i> <i>Ascaris lumbricoides</i> <i>Hymenolepis diminuta</i>
(C)	1	412	Hookworm (1 ovum)
(D-1)	1	159	<i>Endamoeba histolytica</i> (8 mu cysts) <i>Endamoeba coli</i> <i>Endolimax nana</i> <i>Iodamoeba butschlii</i> <i>Giardia lamblia</i> <i>Chilomastix mesnili</i>
(D-2)	2	330	<i>Endamoeba histolytica</i> (8 mu cysts) <i>Endamoeba coli</i> <i>Endolimax nana</i> <i>Giardia lamblia</i> Hookworm <i>Trichuris trichiura</i> <i>Ascaris lumbricoides</i>
(B)	kitchen table		<i>Endamoeba histolytica</i> (12 mu cysts)

human feces although generally considered to be without pathogenic significance were commonly encountered in material from flies in these studies. These parasites include *Endamoeba coli*, *Endolimax nana*, *Trichomonas hominis*, and *Chilomastix mesnili*. *Iodamoeba butschlii* was noted only twice. A summary of these findings is given in tables 1 and 3.

*Flies and evidence of fly breeding noted in connection with these studies.* Due to inherent faults in construction and unsatisfactory maintenance, many of the



native latrines were found to constitute easily accessible sources of food for filth flies and also to be significant foci of fly breeding. The amount of spraying with DDT that was being done was insufficient to control their development adequately. From the public health standpoint, *Chrysomya megacephala* and *Musca sorbens* were observed in studies undertaken by members of the Entomology Laboratory to be the most important species of filth flies on Guam (5). The great prevalence of *Chrysomya megacephala* in latrines had not theretofore been reported, emphasis generally having been placed on its sarcophagous habits. Although entomological reports (6) indicate that this species has been spreading over the tropical Pacific, a majority of the natives here apparently had never seen this fly before the Japanese invasion. Indeed, on the basis of information obtained prior to 1942 relative to flies found on this island, Simmons, Whayne, Anderson and Horak (7) stated that: "It is probable that species of Calliphoridae are also present." This would be a gross understatement of fact today in view of the virtual ubiquity of *Chrysomya megacephala*. Compared with *Chrysomya megacephala*, *Musca sorbens* is very small. Its average body length is only from one quarter to one half of that of the larger fly. It is about one half as large as *Musca domestica* or *Musca vicina*, but it is the most prevalent member of this genus on Guam and the one that has been found to breed in human feces (5).

About 90 per cent of the flies caught in Area A were *Chrysomya megacephala*, with a scattering of *Musca*, *Sarcophaga*, *Lucilia*, *Chrysomya*, *Scholastes* and *Atherigona*. Almost all of the flies caught in Area C at Agana were *Chrysomya megacephala*. Material examined as usual from these flies caught at Agana, as stated earlier, revealed only one hookworm ovum. Although the maintenance of the latrine near which this catch was made could not be considered ideal, the fact that the proprietor of the restaurant took pains to burn out the pit daily with gasoline was probably responsible for the all but complete absence of parasites in the flies encountered there. Garbage disposal was not as satisfactory, being rather sporadic, and there was ample opportunity for fly breeding in the receptacles. Of the 159 flies caught in Area D-1, 135 were *Musca sorbens* and only eight were *Chrysomya megacephala*; this particular catch was made during a period of only two hours. It will be noted in table 3 that no ova were seen in material obtained from these flies, a fact that may well have a causal relationship with the almost complete absence of large flies from this catch. Of the 294 flies caught during a three hour period in Area D-2, 221 were *Musca sorbens* and 68 were *Chrysomya megacephala*. About two months later a trap was again set in this yard directly beside an outdoor table where food was being prepared. During a period of one hour and a half the following flies were caught: *Musca sorbens* 25, *Sarcophaga* 7, *Chrysomya megacephala* 4. The fact that *Endamoeba coli*, *Giardia lamblia*, and demonstrably viable 8 mu cysts of *Endamoeba histolytica* were encountered as a result of this very small catch, has already been noted. It will also be recalled that Area D-2 was the site of the experiments concerning the rôle of flies in the spread of hookworm infection. The absence of demonstrable ova in this particular catch was very probably due to the fact that only a small number of *Chrysomya megacephala* were present. The latrine serving this home

was one of the worst encountered in our studies. The pit was covered by a concrete slab in which were two holes, but only one of them was covered by a box and surrounding housing. The other hole was not sealed so that large numbers of flies passed in and out. If DDT was used at all, it was employed ineffectively.

#### DISCUSSION

The incidence of intestinal parasites on Guam has been shown by studies undertaken in the Parasitology Laboratory of this Unit (1) to be very high. While the transmission of parasites is undoubtedly limited by the use of pit latrines, faulty construction and poor maintenance of many of them provide a readily available food supply for filth flies and favors their breeding. While quantitative estimates are lacking in these studies, observations made over a period of five months all indicate that cysts and ova of various parasites, most significantly of *Endamoeba histolytica* and hookworm, are present in abundance in flies plaguing the inhabitants of certain of the native villages. In spite of this lack of evidence in the form of quantitative measurements, it is our belief that flies are of considerable significance in the spread of amebiasis and hookworm infection on Guam, and probably also of other less important intestinal parasitic diseases.

The relative importance of the different flies caught in these studies cannot be evaluated since they were not segregated according to species before they were permitted to speck the moist gauze. However, the fact seems significant that once in Area D-1 and once in Area D-2 when very few *Chrysomya* were found in the catch, no ova were seen, in spite of the fact that the trap had been set in areas where helminthic infections were considerable. Furthermore, a variety of protozoa were identified that indicated that the flies had had access to fecal material. The conclusion seems justified that the smaller, predominating flies were capable of carrying in the gut and passing with the fly specks the relatively small cysts of protozoa but not the ova of helminths. Such a conclusion is in keeping with the findings of Nicolle to which reference has been made (2).

#### SUMMARY

1. Apparatus used in trapping flies and for collecting and examining their dejecta are described.

2. Data relative to the dissemination of cysts and ova of human intestinal parasites by flies on Guam are presented.

3. The transportation of viable cysts of *Endamoeba histolytica* by flies to a kitchen table in a native home has been demonstrated.

4. A description is given of the transportation of ova of hookworm by flies (presumably from a poorly constructed latrine on the premises) to scraps of food on the ground where they hatch, yielding larvae that complete the free-living portion of the life cycle ending in the infective filariform stage.

5. A note has been made of the more important species of flies encountered. The difficulties of fly control in the face of large scale breeding in latrines that are poorly constructed and maintained are cited.

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On 18 Jan. a re-evaluation was made. The patient had shown no improvement. He continued to run fever ranging from 100 to 104. The cough was quite troublesome, appetite very poor, and constipation continued. Sonorous rales were heard throughout the chest. There was moderate abdominal distention. The spleen was not felt; the liver was not felt but could be percussed 3 cm. below the costal margin and was quite tender. The white blood count was 36,000, of which 52% were eosinophiles. Proctoscopic examination at this time revealed several scattered discrete ulcers, partly covered by yellowish

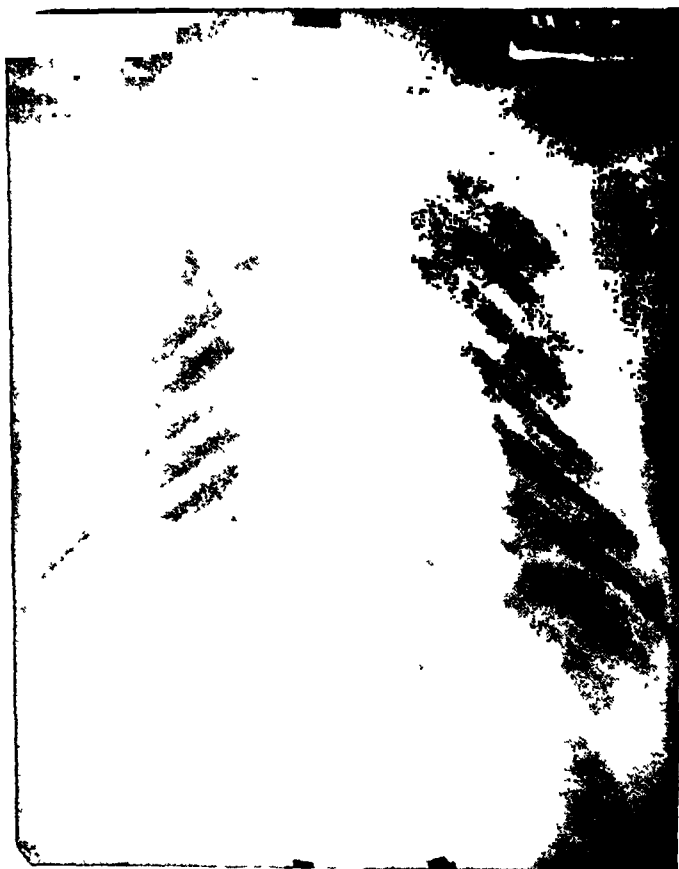


FIG 1 (CASE 1). X-RAY OF CHEST TAKEN 21 JAN. 1945

This portable (supine) chest film shows a diffuse miliary parenchymal infiltration. The film was taken on the 24th day after onset of symptoms.

exudate, in the first 10 cm. of the rectum. Because of the failure of the patient to respond to therapy, it was suggested that the amebiasis might be contributing to his toxic state. Accordingly emetine hydrochloride was started, giving 0.06 Gm. intramuscularly on those alternate days that tartar emetic was not administered.

On 21 Jan. the patient's condition was unchanged. A roentgenogram was made of the chest and showed miliary infiltration throughout both lung fields (fig. 1). At this time penicillin was started on the premise that a secondary bacterial invasion of the lungs might be contributing to his serious condition.

The patient first began to show improvement on 23 Jan. On this day he felt better for the first time since admission to the hospital. At the same time, emetine was discontinued after three doses of 0.06 Gm. each, because the electrocardiogram showed progressive inversion of the T waves in all limb leads; carbarsone was started in dosage of 0.25 Gm. three times daily. Penicillin was discontinued on 24 Jan., after the patient had received 500,000 units. The patient maintained his improvement through 24 and 25 Jan., feeling better, eating better, and looking more comfortable, though the temperature remained unchanged.

On 17 Jan., when the dosage of tartar emetic reached 0.15 Gm., an attack of coughing and vomiting followed the administration of the drug. The same dose was given on 19 Jan., and was followed by a spell of coughing. The next dose, on 21 Jan., was placed in 1000 c.c. of 5% glucose in saline and administered without event. Two days later this was repeated. On 25 Jan. the same procedure was started. However, when 600 c.c. had run in, the patient became very apprehensive and developed marked neuromuscular hyperactivity. The venoclysis was immediately stopped and rebreathing into a paper bag was instituted. In about 15 minutes the patient quieted down completely and felt well. Blood pressure was normal. Five minutes later he suddenly died.

The significant post-mortem findings<sup>3</sup> were as follows: Both lungs were crepitant but were studded throughout with small white moderately firm nodules up to 2 mm. in diameter. The left lung weighed 860 Gm., the right 1100. The liver was extremely large, weighing 3350 Gm. It was also completely studded with pseudotubercles similar to those seen in the lung. The wall of the rectum and sigmoid colon showed similar nodulations. The mesenteric lymph nodes were enlarged. Dissection of the mesenteric veins failed to reveal any adult schistosomes. Microscopic examination of the lungs revealed two types of reaction (fig. 2, a and b). One was the pseudo-tubercle formation which was *seen grossly*. The center of the nodule was *necrotic*, and in some instances showed an intense eosinophilic infiltration. Surrounding this was an area of fibrosis. In the center of some of the nodules could be seen an ovum of *S. japonicum*, in various states of degeneration. The second reaction was a diffuse involvement of the alveoli; many contained a deposit of fibrin with enmeshed cells and a few contained a fluid substance. The cellular elements were chiefly large mononuclears, with a fairly large representation of eosinophiles. Pulmonary aeration was markedly reduced by this process. The liver tissue was diffusely studded with nodules similar to those seen in the lungs (fig. 2 c). These consisted of a small area of necrosis about a schistosomal ovum, a middle zone of cellular reaction including large numbers of eosinophiles and endotheloid cells, and an outer ring of fibrosis. A number of these foci were seen to involve the periportal area. The mesenteric lymph nodes showed similar pseudotubercles which included multi-nucleated giant cells in the cellular zone. One pseudo-tubercle was found in the adrenal gland and one in the appendix.

<sup>3</sup> Autopsy was performed by the 27th Medical Laboratory.

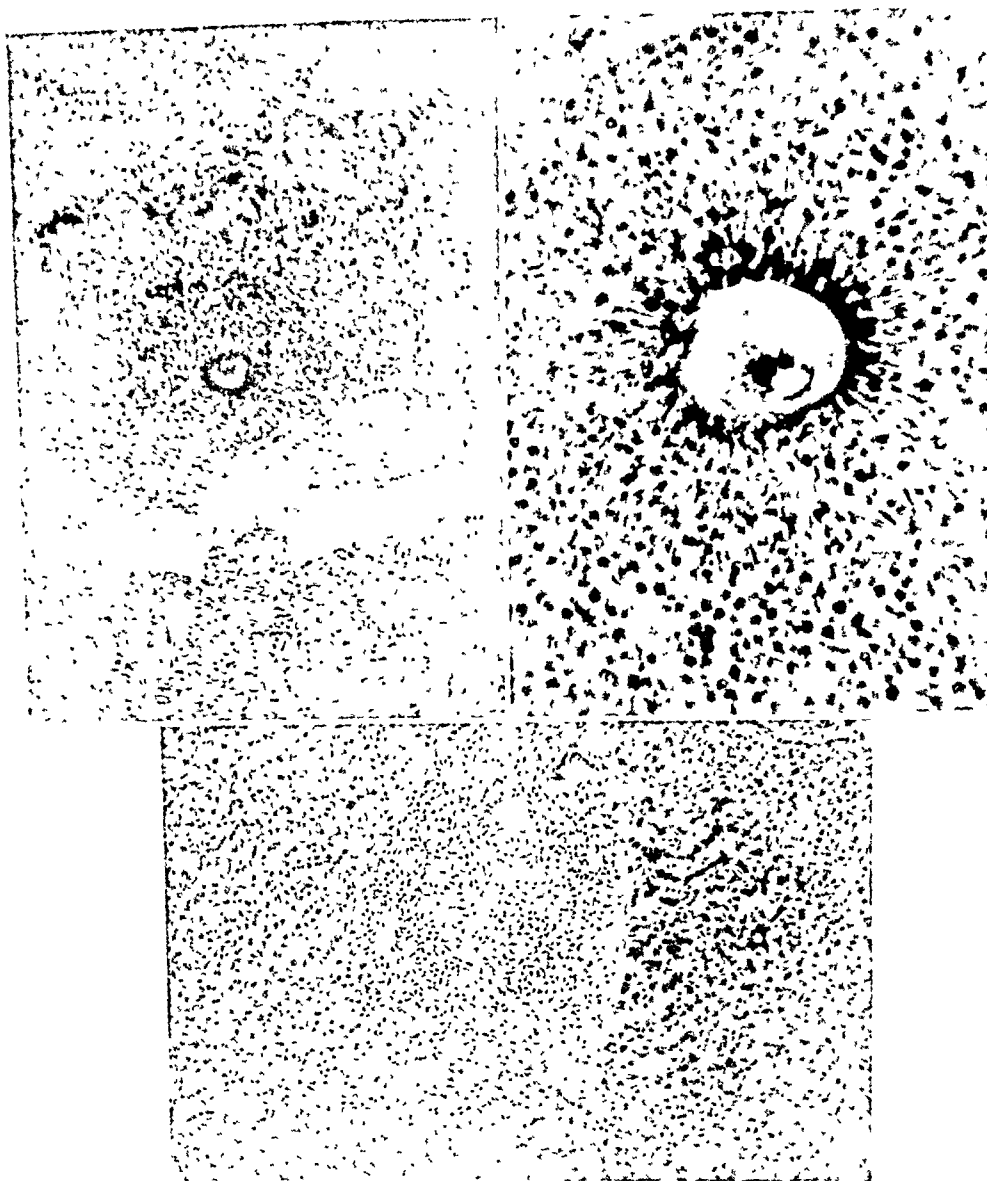


FIG. 2. (CASE 1). PHOTOMICROGRAPHS OF POST-MORTEM SPECIMENS\*

(a) (Upper left) Section of lung  $\times 100$ . This shows both types of the pathology seen in the lungs. There is a miliary granulomatous lesion, in the center of which is a schistosomal ovum. This is surrounded by a zone of cellular reaction with minimal caseation necrosis. The cellular infiltration is greatly polymorphonuclear with a fair number of eosinophiles. The second process involves the alveoli. These are packed with fibrinous exudate and in many there are enmeshed cellular elements; these are chiefly macrophages, with a fairly large representation of eosinophiles.

(b) (Upper right) Section of lung  $\times 440$ . This is a higher magnification of the same section, showing the ovum in the center of the granuloma, and the surrounding cellular reaction.

(c) (Lower) Section of liver  $\times 100$ . This shows a granulomatous lesion in the liver. The central ovum is not seen, but there is necrosis of liver cells with cellular infiltration including one multi-nucleated giant cell.

\*Photomicrography was performed by the 19th General Medical Laboratory.

Clinical diagnoses were: (1) Schistosomiasis, (2) Amebiasis, and (3) Ancylostomiasis.

Anatomic diagnoses were: (1) Diffuse schistosomiasis with pseudo-tubercle formation in the lungs, liver, mesenteric lymph glands, recto-sigmoid colon, adrenal gland, and appendix, and (2) Diffuse lobular pneumonia, secondary to schistosomiasis.

*Case 2.* This 38 year old white soldier came to Leyte, Philippine Islands, on 24 Nov. 1944. During the next 5 or 6 weeks he was occupied with his organization in building bridges. In the course of his occupation he was frequently required to wade and swim in various streams. As per instructions he always bathed in purified water immediately following such exposure, using an anti-septic soap; however the exposure was on some occasions as long as three hours. Around 1 Jan. 1945 the soldier developed a bloody diarrhea which was treated at his battalion aid station and cleared in three days; however, he was left with persistent weakness and anorexia. On 14 Jan. he became very sick, with high fever, dysuria, frequency, and diffuse abdominal discomfort. The following day he was hospitalized in a clearing station; right costo-vertebral tenderness and pyuria were found and a diagnosis of pyelitis made. The patient was given 10 Gm. of sulfadiazine and 130,000 units of penicillin in two days; the dysuria and frequency improved, but he remained very ill and was transferred to a station hospital on 17 Jan. Here the urine was found to be perfectly clear, and examination of the stool on 18 Jan. revealed the presence of ova of *Schistosoma japonicum*. On 20 Jan. the patient was transferred to the 133rd General Hospital. At that time the patient was very sick. He had high fever, often reaching 105 at night, very marked weakness and anorexia, and diffuse abdominal discomfort. Lesser symptoms of nasal congestion and cough had been present since 14 Jan. He denied ever having had "swimmer's itch" or urticaria.

At the time the patient was first seen in this hospital, his oral temperature was 101°F., pulse rate 110, and respiratory rate 22. He was obviously acutely ill, weak, and in discomfort. There was moderate nasal congestion. There were no signs of consolidation in the chest, but sonorous rales were heard bilaterally over the upper half of the chest, more marked in expiration than in inspiration. Cardiac findings were normal. Blood pressure was 104/76-76. The liver edge was felt 2.5 cm. below the costal margin on quiet respiration, descending to 5 cm. on deep inspiration; the liver was soft and very tender. The spleen was not felt but slight tenderness was elicited in the splenic region. Aside from signs of weight loss, the remainder of the physical examination was non-contributory.

Stool examination on 25 Jan. revealed ova of *S. japonicum*, confirming the finding in the previous hospital. Thereafter, on 4, 14, and 21 Febr., no ova nor parasites were found in the stool. On 22 Jan. the red blood cell count was 4,350,000, hemoglobin 85% (Tallqvist), and the white blood cell count 10,500, of which 54% were segmented neutrophils, 28% lymphocytes, and 15% eosinophiles. The white blood cell count was 10,800 on 3 Febr., with 58% eosinophiles, and 15,550 with 40% eosinophiles on 14 Febr. Urinalysis was negative.

Potassium antimony tartrate (tartar emetic) was started in treatment of the schistosomiasis. The patient received 0.03 Gm. intravenously as a 1% solution in distilled water on 21 Jan., 0.06 Gm. on 23 Jan., and 0.09 Gm. on 25 Jan.

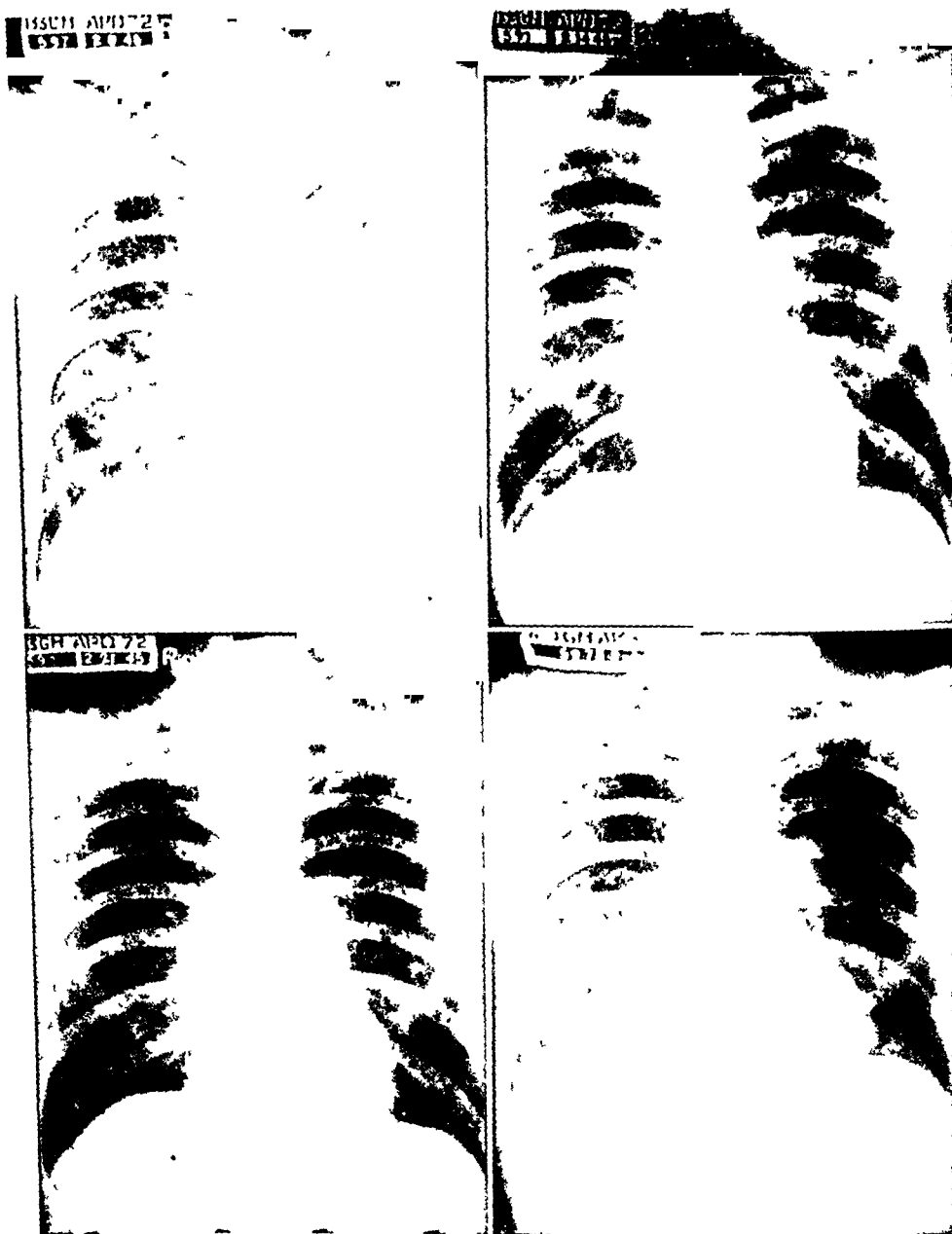


FIG. 3 (CASE 2). SERIAL X-RAY FILMS OF CHEST

(a) (Upper left) 8 Febr 1945. Roentgenogram of the chest taken on the 25th day after onset of acute symptoms shows a diffuse bilateral milary infiltration similar to Case 1 (fig. 1).

(b) (Upper right) 14 Febr. This film taken six days later shows some clearing of the parenchymal infiltration. There is evidence of coalescence of milary areas in the right third anterior interspace.

(c) (Lower left) 21 Febr. One week later there is further clearing. Coalesced areas are visible in the right third anterior interspace and the left fourth anterior interspace.

(d) (Lower right) 4 March 1945. This roentgenogram taken eleven days later and forty-nine days after the onset of acute symptoms shows practically complete clearing of the chest. The coalesced area in the right third anterior interspace is still visible but smaller.



Due to the frequency of cough, nausea, and vomiting in other patients who were receiving the drug, culminating in the death of one (Case 1, v. s.), the use of tartar emetic was discontinued at this time. Beginning 27 Jan. this patient received intramuscular injections of Fuadin on alternate days, getting 1.5 c.c. the first day, 3.5 c.c. the third, and then 5 c.c. for seven doses—a total of 40 c.c. On 2 Febr. he vomited approximately 90 minutes after the fourth dose, but had no reactions at any other time.

For the first two weeks no change could be seen. During this period the oral temperature reached 103 to 105 every day. His complaints did not change, he remained weak, and was very uncomfortable, particularly when the temperature was highest. About 3 Febr., the 15th hospital day, the patient began to feel better. Thereafter he had progressive improvement of all complaints except the cough, and gradually began to regain weight. On 4 Febr. his maximum temperature was 102.4, the first day that it had not reached 103 or higher. Thereafter the temperature fell irregularly by lysis, and on 13 Febr. remained below 99 for 24 hours. For the remainder of his stay in this hospital the temperature varied from 97 to 99.2.

Just about the time that the definite improvement started, the cough became worse around 5 Febr.; it was especially troublesome at night, interfering with sleep, and for the first time became productive of a moderate amount of yellowish mucoid sputum. Examination of the chest revealed the presence of a few scattered inconstant sonorous inspiratory rales bilaterally; fremitus, resonance, and breath sounds were normal. This phase lasted approximately one week, at which time the rales disappeared and the cough became non-productive and less frequent. In another week, around 20 Febr., the coughing ceased. X-rays were made of the chest on 8, 14 and 21 Febr., and 4 March (fig. 3). The patient was evacuated to the rear on 5 March.

The clinical diagnosis was: Schistosomiasis caused by *S. japonicum*, with hepatic, pulmonary, and intestinal involvement.

#### DISCUSSION

Schistosomiasis is a disease produced by the flatworm of the genus *Schistosoma*. Three species are of importance in the production of disease in man, with differing features. The following data were obtained from Circular Letter No. 33, Office of the Surgeon General, dated 2 Febr. 1943 (1). *S. haematobium* is especially prevalent in the Nile Valley and produces mainly genito-urinary symptoms. The adult worms live and lay their eggs in the veins around the bladder. Hematuria, vesical ulcers, and urinary calculi, papillomata, and fistulae occur. *S. mansoni* is found in Africa widely and also in South America and the West Indies; it produces mainly intestinal symptoms. The adult worms live and lay their eggs in the veins around the colon and rectum. Bloodystools, rectal polyps and fistulae, and prolapse of the rectum occur. *S. japonicum* is found in the Far East and produces mainly hepatic symptoms. The adult worms live and lay their eggs in the veins of the small intestines and many eggs are carried to the liver. Bloody stools, hepatic cirrhosis, and splenomegaly occur (1).

The above description was consciously oversimplified. It is well known (2) that the ova are commonly widespread through the body in all three types of schistosomal infestation. In addition to the sites of habitat of the adult worms, ova are found frequently in the liver and lungs, and also in the spleen, appendix, mesenteric lymph glands, brain, heart, kidneys, adrenal glands, and other organs. As the manifestations of the disease are produced essentially by the ova and the reaction of the body to them, the clinical picture will vary with the sites of localization of the ova.

Schistosomiasis caused by *S. japonicum* is divided, according to a standard textbook on tropical disease (2), into three stages: "(1) The incubation period, in which urticarial, pulmonary, and febrile manifestations, may be present. This stage may last a month; (2) that of deposition and extrusion, when the ova appear in bloody mucous stools; and (3) the period of further tissue destruction and proliferation, which may be eventually characterized by cirrhosis of the liver, ascites, cachexia, and death." From this description it would appear that the first stage is occupied by the passage of the schistosome, in their cercarial phase, from the site of entry in the skin, by way of the vascular channels through the lungs to the portal veins, and their development there to adult worms. In our cases, however, we could not delineate such a stage from the one of deposition and extrusion. In the two cases here reported and in others observed (3), schistosomal ova were found in the stool during the time that the urticarial, pulmonary, and febrile manifestations were appearing. From the continuity of the symptoms and signs of pulmonary involvement in case 1, we feel that the pathological process must also have been essentially a continuous one throughout the observed course of the disease. In other words, we feel that the pathological process in the lungs was from its inception the reaction to the localization of ova in those tissues. The impression gained from the textbook description of this disease (2) is that the early pulmonary manifestations are due to passage of cercariae through the pulmonary blood vessels, or allergic and/or toxic factors. However, our findings do not corroborate such a concept.

The pulmonary roentgenographic findings resembled miliary tuberculosis in the two cases presented. The second case showed practically complete clearing of the miliary infiltration on the forty-ninth day after the onset of acute symptoms. The pathological findings are similar to those previously reported in this disease; of especial interest is the localization of ova in the lungs in the earliest phase of the infestation. Our findings do not indicate the ultimate prognosis of the pulmonary involvement. The occurrence of pulmonary fibrosis and even of an Ayerza's syndrome has been described in schistosomiasis caused by *S. Mansoni* and *S. haematobium* (2). The extent of the pseudotubercle formation in case 1 would suggest that this patient would have been left with considerable residual pathology in the lungs had death not supervened. On the other hand, a large amount of alveolar exudate was present, and this could be resorbed. Such a process probably accounted for the relatively rapid radiographic clearing of the lungs in case 2. In addition to the relative extent

of the two processes, many other factors are of importance in prognosis, such as: (1) The timeliness of treatment, whether begun in time to kill the adult worms and the ova before too great irreversible damage has been done; (2) the efficacy of treatment, whether all the adults are killed so that no ova will be deposited in the future; (3) the question of reinfection; and (4) the resistance of the host.

The reaction preceding the death of the patient in case 1 was an unusual one. There was no chill, fever, dyspnea, drop in blood pressure, or change in cardiac rhythm; in 15 minutes there appeared to be complete recovery, but 5 minutes later there was sudden cessation of respiration and cardiac action. The specimen which remained after discontinuance of the venoclysis was unfortunately discarded and could not be checked for impurity. Several other patients on the ward were given injections from the same freshly made batch of the drug at the same time, without any significant reactions. The diluent was a fresh bottle of a commercial saline-glucose preparation. The immediate cause of death is not apparent. However, the temporal juxta-position of drug administration, reaction, and death suggests a cause-and-effect relationship. Fatalities have been reported from the use of antimony products (4), including tartar emetic, but the mode of death in this case is unusual.

#### SUMMARY AND CONCLUSIONS

(1) Two cases of acute schistosomiasis caused by *S. japonicum* are presented. Both showed prominent pulmonary manifestations. One patient died, apparently due to tartar emetic administered intravenously in treatment, and post-mortem examination was made.

(2) The clinical manifestations of pulmonary involvement were cough with expectoration of scant mucoid sputum, chest pain, and scattered rales of no fixed type.

(3) The roentgenographic appearance was that of a miliary tuberculosis. In the patient who recovered there was relatively rapid and almost complete clearance during the period of observation.

(4) Pathologically the lung examined at autopsy was studded throughout with pseudo-tubercles, each about a schistosomal ovum as a nidus. In addition a large amount of alveolar exudate, including cellular elements, was present.

(5) It is suggested that the pulmonary manifestations seen in the first or "incubation" stage of this disease may actually be due to the localization of ova in these tissues.

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# THE DIAGNOSIS OF SCHISTOSOMIASIS MANSONI BY A RECTAL BIOPSY TECHNIQUE<sup>1</sup>

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Though the diagnosis of schistosomiasis *Mansoni* by stool examination may prove extremely easy, there are times when even repeated search will fail to reveal the presence of *Schistosoma mansoni* eggs. This is notoriously true of a chronic or minimal infestation. Moreover, patients who have received treatment with any of the current drugs may show only an occasional egg on repeated examinations of the stools even though the most accurate diagnostic methods have been used. Extensive experience has proved this so on one occasion after another.

Of the several techniques utilized, a modification of the acid-ether concentration has, up to now, appeared to be the most trustworthy in the detection of *Schistosoma* eggs. However, as a follow-up procedure, this method has a certain disadvantage; the eggs become so altered in appearance that it is impossible to determine whether they are viable or not. This is of utmost importance in order to determine the efficacy of the treatment administered.

The other concentration techniques used, in which the live eggs are not destroyed, are lacking in sensitivity and are so time-consuming that they are of no practical value. The miracidium concentration method of Faust and Meloney,<sup>1</sup> as modified by McCorkle *et al.*,<sup>2</sup> seems ideal for showing up the presence of live eggs. However, the time factor limits its applicability as a routine procedure, and the presence of dead eggs cannot be demonstrated.

Other methods of diagnosis, which utilize humoral responses of the body, such as the intradermal reaction to cercarial or adult worm antigen, the flocculation and precipitin tests,<sup>3</sup> may all hold great promise in certain respects, but they fail to prove that the parasite is actually present in the host. In addition, many persons complain that the personal factor may play an important role in the interpretation of results, particularly in the case of the skin tests.

With a view towards determining a definite technique, Ottolina and Atencio,<sup>4</sup> of Venezuela, made a careful study of post mortem material; found that the rectal mucosa is the site of greatest concentration of *Schistosoma mansoni* eggs.

<sup>1</sup> E. C. Faust and H. E. Meloney, Studies on *Schistosoma japonicum*. *Ann. J. Hyg., Mon. Series No. 2*, 1924.

<sup>2</sup> J. K. McCorkle, Modification of Faust-Meloney technique examination of *Schistosoma japonicum*. *U. S. Naval Med. Bul.*, 45: 420-422, 1945.

<sup>3</sup> J. Oliver-González and C. Kreiss Pratt, Skin and precipitin reaction to cercariae from the cercariae and adults of *Schistosoma mansoni*. *P. R. Jour. Pub. Health & Trop. Med.* 27: 357-362, 1945.

<sup>4</sup> C. Ottolina and M. H. Atencio, Nuevas técnicas para el diagnóstico post mortem de la *S. mansoni*. *Rev. Policlínica Caracas*. Vol. 12, No. 72, 1-3, 1946.

and confirmed this finding in patients by obtaining small pieces of the rectal mucosa through a rectoscope. Their technique consisted in removing, with a biopsy forceps, a piece of mucosa about the size of a large grain of rice from the right dorso-ventral rectal fold, located approximately 8 to 10 cm. from the anus. The sample was then placed in 5 cc. of a 4 per cent solution of potassium hydroxide and incubated for three to four hours at 60 to 80°C. The tissue was thus dissolved, and the eggs could be looked for in the sediment left after centrifugation.

To determine the efficacy of the above mentioned method, the writers have put it into practice, with certain modifications, during the past six months. The results have been so satisfactory that they now wish to report on it so that men working in areas, where schistosomiasis is prevalent, may apply it both for diagnosis and for the follow-up of cases. The procedure, as recommended by Ottolina and Atencio, is handicapped by the pre-operative preparation of the patient and by the destruction of the live eggs; the technique as modified by the writers, is the following.

As experience demonstrates that most persons pass stools in the morning, the patient is asked to come to the Outpatient Department in the afternoon. Purgatives and/or enemata are not recommended unless the patient is hospitalized. The rectoscope is introduced according to the usual technique. The first rectal valve is visualized and faces, if present, are removed with a cotton swab. From the edge of the fold, a small piece of the rectal wall is pried loose with a biopsy forceps and the site inspected for any bleeding. This rarely occurs, but if present, may be controlled by pressure with a piece of cotton. The small ulceration left has a whitish base.

Several small pieces of mucosa may be obtained at different sites, but experience has shown that it is not necessary. The selection of the first rectal valve fold for the removal of the sample is important because the patient requires no previous preparation and the biopsy can therefore be performed during his first visit to the clinic. The piece of tissue is then pressed between two microscope slides with the help of Hoffman clamps and examined by low power. The eggs can be easily seen and counted, and the live ones can be readily distinguished from the dead ones. The flattened tissue may also be studied with the help of a magnifying glass, but the sample cannot be studied critically when live, or very few eggs, are present.

In order to determine the value of a rectal mucosa biopsy in the diagnosis of schistosomiasis *Mansoni*, this procedure was applied in a number of cases coming to the Schistosomiasis Clinic of the University Hospital, at San Juan, Puerto Rico. The patients were all adults with a history of having, at one time or another, (a) been rejected from the Armed Forces because of schistosomiasis, (b) come in contact with waters in endemic foci, or (c) suffered from various symptoms that suggested the disease.

A single biopsy was performed on each patient, and a second was done only when deemed necessary. The results were then compared with those obtained by the DeRivas hydrochloric acid-ether concentration technique utilized on

fecal samples of these same patients. The data was also compared in a limited number of cases with those obtained from the intradermal reaction test, when using cercarial antigen. In a few instances, the stools were partly examined by a quantitative dilution technique.

## ANALYSIS OF RESULTS

This study comprised the examination of a total of 138 patients, a large proportion of which had already received the prescribed treatment for schistosomiasis, when the biopsy was performed. Since the main objective pursued in the study was to evaluate this rectal biopsy technique against the DeRivas and intradermal tests, the patients were divided into two groups, as follows: (a) Untreated, comprising those cases that had received no treatment for schisto-

TABLE 1

*Relative results obtained in the rectal mucosa biopsy, De Rivas and intradermal reaction techniques in the diagnosis of Schistosomiasis mansoni*

	UNTREATED GROUP	TREATED GROUP
Total number of cases.....	50	88
Cases positive by rectal mucosa biopsy.....	50	62
Cases negative by rectal mucosa biopsy.....	0	26
Biopsies with living eggs.....	19	18
Biopsies with dead eggs.....	28	68
Cases in which viability was not determined.....	3	2
Number positive by DeRivas test.....	20	16*
Number negative by DeRivas test.....	28	72*
Cases in which DeRivas test was not performed.....	2	0
Average number DeRivas tests to give a positive.....	3.3	10*
Cases positive by skin test.....	11	9*
Cases doubtful by skin test.....	2	1*
Cases negative by skin test.....	3	2*
Cases in which skin test was not done.....	34	76

\* After treatment had been completed.

somiasis at the time of the biopsy. However, a few which had just commenced treatment and had received but one or two injections of the drug have been included in this group. (b) Treated, comprising the cases that, prior to biopsy, had at any time received treatment consisting of a complete series, or several series, of any or various of the antimony compounds employed against *Schistosoma mansoni*. Table 1 shows the results obtained for both of these groups.

The data for the untreated group show that the rectal mucosa biopsy revealed the presence of eggs in 100 per cent of the cases studied, while the DeRivas concentration method demonstrated the presence in only 41 per cent. An average of three fecal samples had to be examined by this last method before a positive result was obtained. The skin test, which was performed in only 16 of the patients, proved positive in 62 per cent of them. In only a single case of this series was it necessary to perform a second rectal mucosa biopsy in order to

obtain a positive result which had proved positive by the DeRivas and kins tests. These data also show that approximately 40 per cent of the patients revealed live eggs in the biopsy as against 60 per cent with only dead ones.

The figures on the treated group reveal that, in spite of treatment, the rectal biopsy was positive in 70 per cent of these cases, while the DeRivas acid-ether concentration technique demonstrated eggs in only 18 per cent of the patients. An average of ten stools examinations had to be performed in order to obtain a positive result. Table 1 also shows that approximately 20 per cent of the biopsies in the treated patients revealed the presence of live eggs, some of which, it was believed, had been but recently laid. Although this aspect of the study is still in a preliminary stage, the writers believe that the rectal mucosa biopsy demonstrated a still active infection in the patients studied, thus suggesting that the

TABLE 2

*Relationship between intradermal, DeRivas and rectal mucosa biopsy techniques in treated persons*

CASE	INTRADERMAL	DE RIVAS*	NUMBER OF EGGS IN BIOPSY
1	negative	negative (10)	1
2	negative	negative (6)	2
3	doubtful	negative (3)	45
4	positive	negative (45)	0
5	positive	negative (26)	0
6	positive	negative (13)	0
7	positive	negative (8)	0
8	positive	negative (5)	1
9	positive	negative (8)	15
10	positive	positive (1 in 5)	32
11	positive	—	142
12	positive	negative (3)	189

\* Number in parentheses refers to number of examinations performed after the end of treatment.

treatment failed to kill all the parasites. The possibility that reinfection may have occurred in at least some of the cases cannot be overlooked, however.

The skin test was performed in 12 treated patients. Table 2 compares the results obtained in this test with those obtained from stool examinations and by the rectal mucosa biopsies. These figures also show that it is very difficult to establish any definite relationship between the various techniques, once treatment has been instituted. This aspect of the problem, however, requires a more detailed study before any definite conclusions can be reached.

Any method of diagnosis should be sufficiently standardized to permit a better evaluation of the results obtained. Although this was rather difficult, the writers have attempted to place the technique of rectoscopic biopsy on a firmer basis by determining the role that the size of the sample of tissue plays in the outcome of the test, since the practitioner will find it extremely hard to obtain constant,



uniform-sized samples. Experience has shown that the size of these samples falls within certain limitations, which the writers have termed large, medium or small according to their measurements when they have been pressed between the slides. These measurements have the following, respective diameters: over one centimeter, from half to one centimeter and less than half a centimeter.

Table 3 illustrates the figures obtained by the writers referable to a single biopsy on each patient and not to variably sized, simultaneous ones on the same patient. The latter would be the most logical way to study this problem, but the writers merely wish at this time to present preliminary observations that may lead to more critical investigations. Table 3 also shows that the number of eggs present may be extremely variable, irrespective of the size of the biopsied sample. Even though the writers believe that the number depends mainly on the degree of infestation rather than on any other single factor, when only diagnosis is concerned, the size of the sample is of very little importance. How-

TABLE 3

*Relationship that size of biopsied sample bears to the number of eggs found in both treated and untreated patients*

LARGE-SIZED BIOPSY		MEDIUM-SIZED BIOPSY		SMALL-SIZED BIOPSY	
Untreated	Treated	Untreated	Treated	Untreated	Treated
4	2	2	1	2	2
6	12	2	7	6	2
8	15	3	11	10	4
13	16	21	17	13	5
45	17	23	19	22	5
50	40	49	22	26	20
57	45	57	55	32	89
66	298	126	62	35	142
69	832	164	100	56	153
140	1000 over	500 over	108	142	163

ever, in order to be on the safe side, it is best to obtain as large a biopsied sample as possible without, of course, jeopardizing the welfare of the patient.

#### DISCUSSION

The work here described fully confirms the observations of Ottolina and Atencio proving that a rectal mucosa biopsy is the most practical method for diagnosing schistosomiasis *Mansoni* infestations. Furthermore, with the modifications introduced by the writers, it is easy and practicable for routine application. This is a method based on the fact that the parasite, *Schistosoma mansoni*, lays its eggs, principally, in the thinner branches of the veins of the sigmoid and rectum of man, a fact corroborated by the writers through finding, in one of the untreated cases, an adult female worm in the part of the submucosa that is usually included in the biopsy. This may be one of the very rare instances in which the parasite has been proved to migrate to such sites to lay its eggs.

or very transparent, and may be easily overlooked by the untrained eye. The least common are the live eggs (fig. 3), which appear just as in the sedimented



FIG. 2. EMPTY SHELLS OF *S. MANSONI* EGGS WITHIN SMALL PSEUDOTUBERCLES

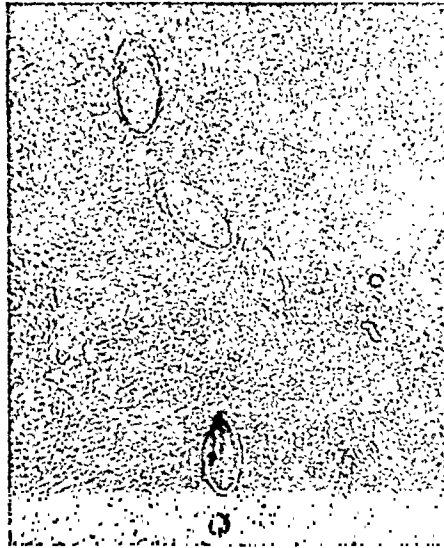


FIG. 3. LIVE EGGS OF *S. MANSONI* AS OBSERVED IN THE RECTAL MUCOSA BIOPSY. MIRACIDIUM CLEARLY OUTLINED. ONE EMPTY SHELL INCLUDED

stools; the miracidium is clearly outlined with the flame cells active. Live eggs are usually found intermingled with the other forms; only rarely are they found alone in a biopsy. Another type of egg (fig. 4), which cannot be placed under



FIG. 4. ROW OF IMMATURE, RECENTLY-LAYED EGGS OF *S. mansoni* AS OBSERVED IN THE RECTAL MUCOSA BIOPSY



FIG. 5. LARGE PSEUDOTUBERCLE WITH NUMEROUS DEAD EGGS, OBTAINED IN A RECTAL MUCOSA BIOPSY

any of the above types, but which is sometimes quite common, appears to have been recently laid, with both poles full of granules that do not show any Brownian movement and a clear central area. The writers believe that these eggs were laid while still immature and therefore failed to develop further. Any of the above types may appear in rows, in masses, or spread singly in the tissue. Outside of the live ones, some may appear encircled in a pseudotubercle, either singly or in masses (fig. 2, 5).

A careful study may give a better clue to the mode by which the eggs of *Schistosoma mansoni* pass through the intestinal wall or to the mechanism by which those that fail to do so and remain stationary, are destroyed and eliminated.

The method here described may also prove of great help in appreciating the therapeutic value of drugs utilized, as well as in determining their effect on the eggs *per se*, as yet a very uncertain problem. Observations to date on treated patients have shown that the egg may remain in the wall of the rectum as long as twenty-one months after the completion of treatment, though repeated examination fail to show them in the stools (table 2). Experience has shown that, during a course of treatment with certain drugs, live eggs continued to be passed for a certain length of time and that these were progressively replaced by dead ones until the latter finally formed the bulk of those passed. By the end of treatment they had disappeared, yet the clinician possesses no way of determining the success of the cure.

Rectal mucosa biopsies may demonstrate the presence of dead eggs, sometimes in extremely large numbers, in these same patients. It is believed that, as long as repeated biopsies continue to show the presence of dead eggs alone, the patient should be considered cured. On the other hand, a return of live ones would suggest that the worms, or at least some of them, had not been destroyed, but just temporarily sterilized. In these cases, however, the possibility of reinfection should not be overlooked.

Another point, which requires careful study and appreciation on the part of the clinician and in which a rectal mucosa biopsy can be of great aid, is that regarding patients who give an old history of having sometimes bathed in an infected body of water during childhood without showing pronounced symptoms of the condition, yet are suspected of harboring the parasite. Several of these cases have been studied by the writers and have usually demonstrated remnants of eggs, or just empty pseudotubercles, in the biopsy while their stools were negative. The procedure to follow in such instances is a series of consecutive biopsies in a search for live eggs. The writers wish to over-emphasize this matter of live eggs, for if they are never encountered, the case in question may be one of extinguished infection.

It is expected that the rectal mucosa biopsy technique, described here, will be given a trial not only in schistosomiasis *Mansoni* but with *japonica* as well.

#### SUMMARY

A method consisting of a rectal mucosa biopsy for the clinical diagnosis of Schistosomiasis *Mansoni*, originally described by Ottolina and Atencio, has been applied by the writers for a period of six months and proved of extreme effective-

ness. Several modifications, which make it easy and practical for routine applications, have been introduced to it. The basis for evaluating this technique is founded on a comparison of the results, obtained from its use, and those derived from stool examinations by the acid-ether concentration and the intradermal reaction tests in both treated and untreated cases of schistosomiasis.

In the untreated cases this rectal biopsy technique proved 100 per cent effective, while the acid-ether concentration and intradermal reaction tests were found positive in only 40 per cent of the same patients and in 62 per cent of the 16 patients that were tested. This biopsy technique was also positive in 70 per cent of the treated cases; the acid-ether concentration in only 18 per cent after an average of ten consecutive examinations had been performed. The intradermal reaction proved quite variable in 12 members of this group, the final results bearing no relationship to those obtained with the biopsy technique.

Besides simplifying the method for obtaining a rectal mucosa sample, the modifications introduced by the writers into the original Ottolina and Atencio method will not only permit its use for a more careful study of schistosomiasis, but also for the elucidation of many, as yet, obscure problems pertaining to it.

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# EFFECT OF SULFADIAZINE ON CHOLERA\*

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Following the successful treatment of bacillary dysentery with sulfaguanidine, a few authors have used this drug in cholera with encouraging results (1, 6, 7). As sulfadiazine has been shown to be effective for bacillary dysentery and allied conditions (4, 5), it seems worthwhile to try this sulfa-derivative on cholera. The cholera epidemic in Foochow in the late summer of 1944 afforded an opportunity for such a clinical trial.

## METHOD OF STUDY

The present study was carried out in a separate ward in the Foochow Cooperative Hospital, which became an isolation hospital during the cholera epidemic. Ten cholera patients were given sulfadiazine in addition to saline infusions, while ten other patients receiving only saline infusions served as control. Only adult patients seen within 48 hours after the onset of illness were used in this study. In each case the diagnosis of cholera was confirmed by positive stool culture. Stool culture was made on admission and repeated once or twice during the patient's stay in the hospital.

As frequent vomiting interfered with oral administration of sulfadiazine at the time of admission, sodium sulfadiazine (a single dose of 3 gm. in 5% solution) was given intravenously together with normal saline and 2% sodium bicarbonate solution. As soon as vomiting became less frequent, oral sulfadiazine therapy (6 gm. daily) was instituted until the subsidence of diarrhea.

The patients were encouraged to drink 50-100 cc. of 0.5-1% sodium bicarbonate solution every half an hour. Three to five liters of fluid could be taken orally even during the collapse stage. In this way, 20-40 gm. of sodium bicarbonate were also provided to counteract acidosis.

## RESULTS

The clinical data of the two groups of patients with and without sulfadiazine therapy respectively are shown in tables 1 and 2.

The fatal case in the control group was due to lobar pneumonia developed on the 4th day of disease. One case of parotitis also occurred in the control group. Urticarial rash appeared in a sulfadiazine-treated patient during convalescence. Similar cutaneous eruption was seen in a control case. Skin rash occurred rather frequently in this epidemic, having no relation with the use of sulfonamides. Suppression of urine was common during the collapse stage but never persisted for more than 48 hours in the cases under observation. No renal complication was encountered in either group.

\* The sulfadiazine used in this study was kindly supplied by Lederle Laboratories, Inc., New York.

It was not practicable to keep the patients in hospital until the stool culture became negative. Stool cultures taken on the 6th, 7th or 8th day of disease were still positive in 8 out of 10 cases with sulfadiazine treatment and in 7 out of 8 control cases.

TABLE 1  
*Clinical data of patients with sulfadiazine therapy*

HOSPITAL NUMBER	SEX	AGE	HOURS ILL BEFORE TREATMENT	DURATION OF DIARRHEA*	TOTAL SALINE INFUSED	RESULTS
				days	cc.	
259	M	34	3	2	5,000	Recovered
295	M	49	11	3	6,500	Recovered
334	F	33	13	3	11,000	Recovered
350	M	20	12	6	1,500	Recovered
355	F	35	41	5	1,500	Recovered
356	M	21	21	6	11,200	Recovered
370	F	32	24	7	6,000	Recovered
381	M	22	11	5	3,000	Recovered
382	M	35	12	5	11,500	Recovered
402	M	50	14	4	5,500	Recovered
Average.....		33.1	16.2	4.6	6,270	

TABLE 2  
*Clinical data of patients without sulfadiazine therapy (control)*

HOSPITAL NUMBER	SEX	AGE	HOURS ILL BEFORE TREATMENT	DURATION OF DIARRHEA*	TOTAL SALINE INFUSED	RESULTS
				days	cc.	
255	M	46	3	6	6,200	Recovered
290	M	34	24	14	10,000	Recovered
323	M	23	16	6	3,500	Recovered
342	F	23	25	6	4,500	Recovered
343	M	30	28	6	14,500	Recovered
357	M	38	48	9	9,000	Recovered
371	F	33	8	5	4,000	Recovered
380	M	48	27	—	5,000	Died
383	F	42	10	6	13,500	Recovered
401	M	58	19	6	3,500	Recovered
Average.....		37.5	20.8	7.1	7,370	

\* From the onset of diarrhea until the number of stools was reduced to two or less in 24 hours.

#### DISCUSSION

Laboratory studies have shown that sulfadiazine has good *in vitro* and *in vivo* effects on the cholera vibrio (3, 8). As clinical use has also demonstrated the efficacy of sulfadiazine in acute diarrheal diseases (4, 5), this drug may be ex-

pected to have beneficial effect on cholera. In the present series, the average duration of diarrhea is shorter and the amount of saline infused is smaller in the group of patients with sulfadiazine treatment as compared with the control. This is in accord with the favorable results obtained with sulfaguanidine. However, the number of cases studied is too small to warrant any definite conclusion. Sulfadiazine, being readily absorbable, has the advantage over sulfaguanidine in the prevention of complications due to secondary bacterial invasion such as pneumonia and parotitis. It is to be noted that no renal complication attributable to sulfadiazine therapy has been observed in this series. Recently Fox (2) reported the successful treatment of burn shock with sodium lactate solution by mouth. We have shown that during the collapse stage of cholera a fairly large amount of fluid and alkalies can be given by the oral route. If oral administration could be pushed energetically but cautiously, it should be possible to reduce the number of intravenous infusions and thus lessen the burden of the usually overworked medical staff of a cholera hospital.

## SUMMARY

1. In a cholera epidemic, 10 patients were given sulfadiazine in addition to saline infusions with 10 other patients without sulfadiazine treatment as control.
2. The average duration of diarrhea of the sulfadiazine-treated patients (4.6 days) is shorter than that of the controls (7.1 days).
3. One case of lobar pneumonia resulting in death and one case of parotitis occurred among the patients without sulfadiazine therapy.
4. Oral administration of fluid and alkalies in the treatment of cholera has been emphasized.

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## SULFONAMIDE DRUGS IN THE TREATMENT OF CHOLERA\*

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In the treatment of cholera, the main emphasis has been laid on the replenishment of fluid and salts lost through purge and emesis. The treatment of the infection itself has received rather scanty attention. Some authors contend that cholera is a self-limited disease and that if the patient can be tided over the critical period by symptomatic treatment the infection will subside by itself. Be that as it may, antibacterial measures promptly instituted in conjunction with saline infusion may be expected to shorten the course and lower the mortality.

The experiments of Griffiths (4) and Sadusk and Oswald (10) have shown that the sulfonamides have favorable *in vitro* and *in vivo* effects on the cholera vibrio. Though the reports of Pasricha (7) and Carruthers (1), using sulfapyridine and sulfaguanidine respectively, are not encouraging, those of Chopra et al (2), Huang Joo-se (6) and Chu and Huang (3) have indicated that both sulfaguanidine and sulfadiazine have good therapeutic value in the treatment of cholera. The present report is a further clinical study of this problem.

### CLINICAL MATERIAL AND METHOD OF STUDY

The present study was made in the Hsiao-Lung-K'an Emergency Hospital during the cholera epidemic in Chungking in the summer of 1945. Only adult patients receiving treatment within 72 hours after the onset of disease were included in this study. The patients were divided into three groups, receiving practically identical treatment except for the sulfonamide therapy. Of the 79 patients under study, 25 were in the group with sulfaguanidine treatment, 25 in the group with sulfadiazine treatment and 29 in the control group.

The control cases were given saline infusion and symptomatic treatment only. For the patients with sulfaguanidine treatment, besides fluid replenishment measures, sulfaguanidine 5 gm. initially and 2.5 gm. every 4 hours was given until the number of stools in 24 hours was reduced to two or less. The sulfadiazine-treated patients were given 50 cc. of 5 per cent sodium sulfadiazine solution intravenously followed by oral administration of sulfadiazine 1 gm. every 4 hours until the diarrhea subsided. In one instance intravenous sodium sulfadiazine alone was used. Sulfonamide concentration in the stool as well as in the blood was determined in some cases during treatment.

At the time of admission practically all the patients were markedly dehydrated and collapsed, requiring immediate intravenous infusion. Physiological salt solution was used for correcting dehydration. Sodium bicarbonate 2 per cent

\* The sulfadiazine and sulfaguanidine used in this study were kindly supplied by Lederle Laboratories, Inc., New York.

and glucose 10-20 per cent solutions were also used whenever indicated. Intramedullary infusion using the needle of Tocantins et al (11)<sup>1</sup> was made in a few cases of severe collapse when venepuncture could not be successfully performed. The amount of fluid infused and the frequency of infusions were guided by the specific gravity of blood determined by the copper sulfate method of Phillips, Van Slyke and collaborators (8). Following the suggestion of Chu and Huang (3), the patients were encouraged to drink one per cent sodium bicarbonate solution as a supplementary measure for dehydration and counter-acting acidosis. A considerable amount (2 to 5 liters in 24 hours) could easily be taken even during the stages of evacuation and collapse.

Stool specimens for culture were obtained by means of a glass rectal tube. The tube is 25 cm. long and has an inner diameter of 7 mm. It is closed at one end but with an opening 1 cm. above the blunt, round tip. It was sterilized in the autoclave before use. An uncontaminated specimen of liquid stool could be obtained directly from the rectum for inoculation of alkaline peptone media. Boric acid preservative fluid (12) was also used in occasions when culture had to be delayed. The cultures were made at 2-5 day intervals during the patient's stay in the hospital.

#### ANALYSIS OF CLINICAL AND LABORATORY DATA

*General data.* The average age of the patients, number of hours ill before treatment, percentage having had anti-cholera inoculation and the average value of blood specific gravity at the time of admission of the three groups of patients are given in table 1.

*Fatal cases.* There are two fatal cases in this series, one in the control group and the other in the group with sulfaguanidine treatment. The fatality in the control group was a case of rapidly developing uremia resulting in coma and death in spite of energetic treatment. Among the sulfaguanidine-treated patients death occurred in a 67-year old man during convalescence. Old age and debility were probably responsible.

*Complications.* Besides the case of uremia ending in death, no other renal complication was observed in the control as well as the sulfonamide-treated patients. Though pyrogenic reaction following intravenous infusion occurred frequently,<sup>2</sup> hyperpyrexia or "cholera-typhoid" was not encountered. Generalized urticarial rash occurred in a patient after intravenous injection of sodium sulfadiazine. Purpuric eruption with gingival bleeding and extravasation into the buccal mucosa was observed in a patient under sulfaguanidine treatment. These conditions rapidly subsided after discontinuance of the drugs.

*Duration of diarrhea.* The diarrhea was considered subsided when the number of stools was reduced to two or less in 24 hours. With a few exceptions the stool became fecal in character by that time. The average durations from the start

<sup>1</sup> The needle was brought by Dr. H. A. Reiman from Philadelphia for use in the treatment of cholera.

<sup>2</sup> To meet the emergency of preparing saline solution in large quantity, distilled water which was a by-product of waste steam in a nearby factory was used without re-distillation.

of treatment to the subsidence of diarrhea of the three groups respectively are given in table 2. The average duration of diarrhea of either sulfaguanidine- or sulfadiazine-treated group is shorter than that of the control and the difference is more than three times its standard error. Though the difference is less than 2 days, it is statistically significant.

TABLE 1  
*General data of the three groups of cholera patients*

GROUP	NUMBER OF CASES	AVERAGE AGE	HOURS ILL BEFORE TREATMENT	ANTICHOLERA INOCULATION	AVERAGE BLOOD SPECIFIC GRAVITY ON ADMISSION
				%	
SQ	25	33.8	29.5	12	1.0655
SD	25	30.5	24.4	20	1.0664
Control	29	25.7	22.5	24	1.0667

SQ = sulfaguanidine; SD = sulfadiazine.

TABLE 2  
*Comparison of the interval from the start of treatment to the subsidence of diarrhea (days)*

GROUP	MEAN	STANDARD ERROR (S.E.) OF MEAN	DIFFERENCE FROM CONTROL	S.E. OF DIFFERENCE	RATIO OF DIFFERENCE TO ITS S.E.
SQ	2.38	0.23	1.41	0.44	3.23
SD	2.12	0.21	1.67	0.43	3.89
Control	3.79	0.37	—	—	—

TABLE 3  
*Comparison of the total amounts of intravenous fluid used (cc.)*

GROUP	MEAN	STANDARD ERRORS (S.E.) OF MEAN	DIFFERENCE FROM CONTROL	S.E. OF DIFFERENCE	RATIO OF DIFFERENCE TO ITS S.E.
SQ	2413	290	1944	683	2.85
SD	3828	714	529	944	0.56
Control	4357	618	—	—	—

*Amount of intravenous fluid used.* Since the amount and frequency of intravenous (and intramedullary) infusions were controlled by repeated determinations of blood specific gravity, these data can be used for comparison. The average total amounts of intravenous fluid used by these three groups are calculated and compared (table 3). The sulfaguanidine-treated patients on the average required much less intravenous saline as compared with the control and the difference is significant. The sulfadiazine-treated patients, on the other hand, also used less saline but the difference is only 0.56 of its standard error.

It may be argued that the amount of intravenous fluid given before the drug has sufficient time to exert its effect should vary only within the errors attributable to chance, whereas that given later on would be a more reliable index of the efficacy of treatment. For this reason, the amount of intravenous fluid given on the first day after admission and that given on subsequent days are calculated separately (tables 4 and 5). In the former case, the differences between the groups with sulfonamide treatment and the control are about equal to their respective standard errors and are therefore probably sampling errors, while in the latter case, the differences are 2 or 3 times their standard errors and therefore cannot be attributed to chance.

TABLE 4  
*Comparison of the amounts of intravenous fluid (cc.) given on the first day of hospitalization*

GROUP	MEAN	STANDARD ERROR (S.E.) OF MEAN	DIFFERENCE FROM CONTROL	S.E. OF DIFFER- ENCE	RATIO OF DIFFERENCE TO ITS S.E.
SQ	2150	223	350	326	1.07
SD	3128	526	628	577	1.09
Control	2500	238	—	—	—

TABLE 5  
*Comparison of the amounts of intravenous fluid (cc.) given after the first day of hospitalization*

GROUP	MEAN	STANDARD ERRORS (S.E.) OF MEAN	DIFFERENCE FROM CONTROL	S.E. OF DIFFER- ENCE	RATIO OF DIFFERENCE TO ITS S.E.
SQ	262	152	1595	505	3.16
SD	700	259	1157	547	2.12
Control	1857	482	—	—	—

*Result of rectal tube cultures.* The diagnosis of cholera in every case of this series was proven by the isolation of cholera vibrio from the stool. As the follow-up cultures were made at 2-5 day intervals, it is not possible, basing on these data, to determine exactly how soon the cholera vibrios disappear from the stool. However, at the end of one week after the start of treatment, 10 of 21 sulfaguanidine-treated patients (47.6%), 11 of 21 sulfadiazine-treated patients (52.4%) and 10 of 22 control patients (45.5%) already had negative rectal tube cultures. Among the patients under observation, none had positive stool culture after the 13th day of disease. In most instances, cholera vibrio could still be isolated from the rectal tube cultures for varying periods after the diarrhea had subsided. This occurred in the control as well as in the sulfonamide-treated cases.

*Sulfonamide concentration in stool and blood.* The sulfaguanidine concentration in the blood ranges from 0.8 to 4.2 mg. per 100 cc., while that in the rice-water stool ranges from 25 to 40 mg. per 100 cc. The drug concentration in the stool during the period of diarrhea is rather low, probably due to the

frequent and copious evacuations. Part of the drug may also be lost in the vomitus.

The sulfadiazine level in the blood ranges from 4 to 10 mg. per 100 cc., while that in the liquid stool ranges from 3 to 6 mg. per 100 cc. Considering the tremendous loss of fluid through the intestinal tract, it may be surmised that a large portion of the sulfadiazine in the blood may pass over into the stool during the stage of evacuation. In order to prove this point, one patient was given sodium sulfadiazine intravenously in doses of 2.5 gm. twice daily without oral administration of sulfonamide drugs. Simultaneous determinations six hours after the first intravenous dose showed that the sulfadiazine concentration was 4 mg. per 100 cc. in the blood and 3 mg. per 100 cc. in the stool. Determinations made on the next day showed that the concentration in the blood and in the stool were 6 mg. and 5 mg. per 100 cc. respectively.

#### DISCUSSION

From the above analysis, sulfaguanidine and sulfadiazine in the dosage employed have been shown to have favorable effect on the clinical course of cholera in that the duration of diarrhea is shortened and the amount of intravenous fluid used is reduced. The differences between the groups with sulfonamide treatment and the control are statistically significant. However, there is no significant difference between the clinical results produced by these two sulfonamides.

The sulfonamides used in this study exert no remarkable effect on the time of disappearance of the cholera vibrios from the patients' stool. It is interesting to note that the use of streptomycin in cholera also produces no marked influence on the results of the stool cultures (9). This is in contrast to the striking results produced in the treatment of bacillary dysentery with sulfonamide drugs (5). The *in vitro* experiments of Sadusk and Oswald (10) have shown that sulfadiazine and sulfaguanidine in concentration of 1-2 mg. per 100 cc. is sufficient to inhibit the growth of *V. cholerae*, while a much higher concentration (100-200 mg. per 100 cc.) is required to produce bactericidal effect. In the present study, determinations of the free sulfadiazine and sulfaguanidine concentrations in the stool show that only bacteriostatic concentration has been attained. When the drug concentration of the stool specimen is diluted in the culture medium, growth of the cholera vibrios may still be possible. Whether sulfaguanidine in bigger doses and for a longer time can produce bactericidal concentration in the stool and thereby shorten the period of convalescent carrier state remains to be studied.

It is interesting to note that no renal complication attributable to sulfadiazine therapy has been observed in the present study as well as in the small series of cases reported by Chu and Huang (3). We have demonstrated that sulfadiazine passes readily from the blood into the stool during the period of copious diarrhea when suppression of urine usually occurs. Consequently the drug concentration in the blood has never been found to reach a very high level. If alkalis are used liberally at the same time there would be no danger of increasing the incidence of uremia, the much dreaded complication of cholera.

Sadusk and Oswald (10) suggested that since succinyl sulfathiazole is hydrolyzed into sulfathiazole and succinic acid in the intestinal tract, the combination of bacteriostatic effect and acid reaction would give excellent therapeutic results. This sulfa-derivative is certainly worthy of a clinical trial in cholera.

#### SUMMARY

1. In a cholera epidemic, a study of the therapeutic effect of sulfonamide drugs has been made in 25 adult patients treated with sulfaguanidine, 25 with sulfadiazine and 29 without sulfonamides as control.
2. One death occurred in the control group and one in the group with sulfaguanidine treatment.
3. The average duration of diarrhea is shortened and the average amount of intravenous saline given is reduced in the sulfonamide-treated group as compared with the control. The differences are statistically significant.
4. There is no significant difference between the therapeutic results produced by these two sulfonamide drugs.
5. No striking effect of the sulfonamides on the time of disappearance of cholera vibrios from the stool has been observed.
6. With the dosage used in this study, only bacteriostatic concentration of the sulfonamides has been found in the stool of the patients under treatment.

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# TREATMENT OF BUBONIC PLAGUE WITH SULFADIAZINE\*

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Fukien Province in Southeastern China which was invaded by plague in 1894 has remained an important endemic center (19, 20). At first the infected area seemed to be limited to districts near the coast but since the Sino-Japanese War, probably due to increased inland traffic, the disease began to appear in localities farther inland and even spread to the neighboring provinces (5). Outbreaks of epidemic proportions occurred from time to time. Curative work, no less important than preventive measures, had become an urgent problem confronting the local health authorities.

Since the introduction of the sulfonamides, a number of laboratory studies as well as clinical trials have been made with various sulfa-derivatives in the treatment of plague (1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 14, 15, 17, 18). Though the results with sulfanilamide and sulfapyridine were not uniformly favorable, the extensive field trials by Sokhey and his associates have proved beyond doubt the therapeutic efficacy of sulfathiazole (15). More recent experiments and clinical trials seem to indicate that sulfadiazine might be more effective than sulfathiazole (4, 8, 18).

In view of the pressing demand for an effective therapeutic agent for plague, we made a special trip to Fukien in the summer of 1944 for the purpose of testing the value of sulfadiazine in this disease. Foochow, being a populous city, was chosen for making this study because more patients and better clinical facilities were available.

## CLINICAL FACILITIES AND METHOD OF STUDY

The present study was carried out in a special establishment for the treatment of plague patients annexed to the Foochow Cooperative Hospital. This plague unit consists of an out-patient clinic, a general ward of 18 beds and a separate ward with 3 cubicles for pneumonic cases. From the beginning of June up to the commencement of our study (July 18), 109 plague patients had been admitted, of whom 56 died. The high case fatality rate (51.4%) was probably due to inadequate treatment, because before our arrival sulfonamide preparations as well as antiplague serum were scarce and rather expensive.

From July 18 to Sept. 6, 27 patients were admitted, 25 of whom had bubonic plague. The remaining two, suffering from pneumonic and septicemic plague respectively, were admitted *in extremis* and died before treatment

\* The expenses for the trip were defrayed from a grant by Lederle Laboratories, Inc., New York. The sulfadiazine used in this study was also kindly supplied by this firm.

<sup>1</sup> We are indebted to Dr. Y. C. Wang, Superintendent of Foochow Cooperative Hospital for permission to use the hospital facilities.



was started; therefore they will not be included in the following presentation. Originally we planned to divide the cases into three groups with sulfadiazine, sulfathiazole and serum treatment respectively. But on account of the limited number of patients, the plan had to be dropped and the majority were treated with sulfadiazine only.

The diagnosis of plague was established by both bubo and blood cultures made at the time of admission. The material obtained from bubo aspiration was studied in stained smears as well as cultured on blood agar slants. Blood cultures were made by inoculating  $\frac{1}{2}$  cc. of blood into broth and on agar slants. The bacteriological work was done by Dr. C. Y. Wu in the Provincial Hygienic Laboratory nearby.

The dosage of sulfadiazine (and sulfathiazole) for adults was 4 gm. initially followed by 1 gm. every 4 hours. In a few severely affected patients, larger doses were employed. The sulfonamide treatment was continued until the temperature had been normal for two or more days. Sodium bicarbonate in equal or larger amounts was given together with the sulfonamides. It was not possible to determine the sulfonamide concentration in the blood but urine and blood examinations were made at frequent intervals during the treatment to guard against toxic reactions.

The antiplague serum used in this study was immune horse serum prepared by the Central Epidemic Prevention Bureau in Kunming and supplied to us through the courtesy of Dr. C. H. Yiu, the Provincial Health Commissioner of Fukien.

#### RESULTS

Among the 25 cases of bubonic plague in the present series, 16 were treated with sulfadiazine alone, 3 with sulfadiazine and serum, 4 at first with sulfathiazole and serum but later with sulfadiazine and 2 with sulfathiazole alone. The clinical data of these 25 patients are given in table 1.

*Influence of promptness of treatment on clinical results.* In the present series, all the cases admitted on the first or second day of illness responded promptly to treatment. Mortality occurred only among those admitted on or after the third day of illness. A study of the temperature charts of the recovered cases clearly shows that the earlier the treatment is started, the speedier is the recovery. In patients receiving sulfonamide therapy within 24 hours after onset, the temperature came down by crisis within 12 hours of treatment. In those admitted on the second day of illness (with one exception), the temperature became normal after 24 hours of treatment. If treatment was delayed for three or more days after onset, it took 72 or more hours of treatment before the fever completely subsided. The temperature charts of representative cases are given for illustration.

*Local response to sulfonamide therapy.* In the majority of cases, during sulfonamide therapy the bubo became smaller, softer and less tender with simultaneous amelioration of local redness and swelling. However, in five cases, soon after the institution of treatment, the bubo suddenly became markedly increased in size and the local reaction also became very much exaggerated.

Two of these patients died and the other three had to run a stormy and protracted course to attain recovery. In only four cases of the present series marked suppuration of the bubo resulted, requiring incision and drainage.

TABLE 1  
*Clinical data of 25 cases of bubonic plague and the results of treatment*

HOSPITAL NO.	SEX	AGE	DAYS ILL BEFORE ADMISSION	ANTI-PLAGUE INOCULATION	BUBOES	BUBO SMEAR	BUBO CULTURE	BLOOD CULTURE	SULFADIAZINE gm.	SULFATHIAZOLE gm.	ANTI-PLAGUE SERUM cc.	RESULT
96	M	36	2	none	Inguinal							
107	M	7	1	none	Femoral	+	+	+	43	23	140	Recovered
108	F	25	3	none	Inguinal	+	+	+	12			Recovered
109	M	14	8	none	Cervical, submaxillary	+	+	+	73	38	80	Recovered
111	M	9	8	yes	Femoral				146		40	Recovered
113	M	25	3	none	inguinal	+	+	+				Recovered
114	F	15	3	yes	Femoral				17			Recovered
117	F	56	3	yes	Femoral	+	+	+				Died
118	F	20	2	yes	Femoral, inguinal	+	0	+	45	29	120	Recovered
119	F	57	3	none	Axillary	+	+	+	30	25		Recovered
120	F	28	2	none	Femoral, inguinal	0	+	+	16			Recovered
121	F	53	2	none	Femoral	+	+	+	69			Recovered
122	M	33	2	none	Inguinal	+	+	+				Recovered
124	M	12	5	none	Cervical	+	+	+	56	47	80	Died
126	M	20	3	none	Cervical	+	+	+	62			Recovered
129	F	12	1	none	Femoral, inguinal	0	+	0	11			Recovered
130	F	38	4	none	Axillary	+	+	0	34			Recovered
131	M	63	3	none	Cervical, femoral	0	+	0	17			Recovered
132	F	30	2	none	Inguinal	+	+	0	33			Recovered
133	F	25	2	yes	Femoral	+	0	0	68			Recovered
134	F	66	3	none	Inguinal	+	+	+	24			Recovered
136	M	10	1	none	Inguinal	+	+	+	20			Recovered
137	M	17	3	none	Femoral	+	+	+	22			Recovered
138	F	8	2	none	Femoral	0	+	+	33			Recovered
139	M	16	1	none	Inguinal	+	+	+	12			Died
				none	Femoral	0	+	+	28			Recovered
						+	+	+	10			Recovered
						+	+	+	12			Recovered

In six cases of this series, we found on the skin drained by lymphatics in the region of the bubo small vesicular and pustular lesions, from which *P. pestis* could be cultured. These manifestations represented presumably the initial lesions produced by the bites of infected fleas. They rapidly healed up during treatment with or without local application of sulfonamides.

*Effect on the causative organism.* Culture of the aspirated material from bubo

was positive in 22 out of 24 cases (91.7%), while direct smear showed bipolar-stained bacilli in 20 out of 25 cases (80.0%). Blood culture was positive in 19 out of 25 cases (76.0%). In a few cases where both blood and bubo cultures were repeated during the course of treatment, it was found that when the temperature became normal the blood culture also became negative, whereas plague bacilli could still be isolated from the bubo for varying periods after the fever had subsided.

*A case of repeated relapses.* The fact that the sulfonamides readily control the bacteremia while leaving viable plague bacilli in the buboes for relatively long periods suggests the possibility of relapses. Such relapses may actually have occurred in the following case.

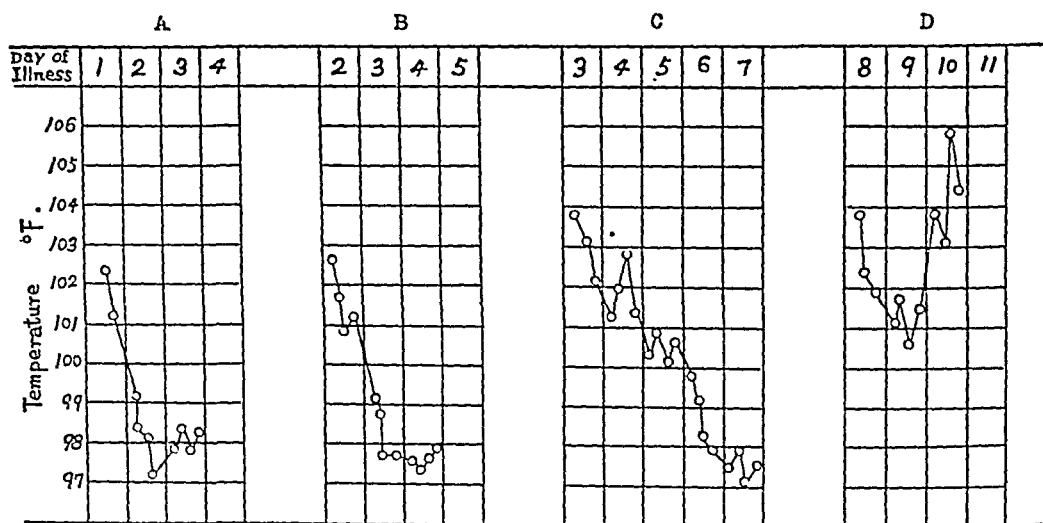


FIG. 1

- A. Treatment started on the first day of illness. Recovered. (H. N. 107)  
 B. Treatment started on the second day of illness. Recovered. (H. N. 138)  
 C. Treatment started on the third day of illness. Recovered. (H. N. 117)  
 D. Treatment started on the eighth day of illness. Died. (H. N. 111)

*Case 1.* H. N. 109. An undernourished lad, 14 years old, was admitted in a semicomatose condition after being ill for 4 days with high fever and large buboes over the right cervical and left submaxillary regions. Sulfadiazine 3 gm. initially followed by 1 gm. every 4 hours was given. In addition, 40 cc. of anti-plague serum were administered intravenously on the first day of hospitalization. There was steady improvement in the general condition. On the 7th day after admission, the blood culture became negative though the patient still ran a low-grade fever and the buboes remained large and tender. Next day, the dosage of sulfadiazine was cut down to 4 gm. a day. Two days later a large abscess appeared in the left axilla. Culture of the aspirated material was positive for *P. pestis*. *Pari passu* the fever became higher and septic in character. The dosage of sulfadiazine was again increased to 6 gm. a day. The abscess was incised and instilled with 0.5% sodium sulfadiazine solution. After a few days the fever subsided and the axillary abscess promptly healed up, though

the cervical and submaxillary buboes remained firm and tender. Sulfadiazine therapy was discontinued 3 days after the temperature had become normal. At this time, the patient had been under treatment for 20 days and had received 109 gm. of sulfadiazine.

However, two days after stopping the drug, patient again ran a low-grade fever and a new bubo appeared in the left inguinal region. Culture of the aspirated material grew *P. pestis*. Sulfonamides were purposely withheld to see if a serious relapse could occur. The inguinal bubo subsided in a few days without treatment. But soon another tender mass appeared over the left side of the neck. Aspiration obtained purulent material. Direct smear showed many pus cells and a few bipolar-stained bacilli, some of which appeared to be phagocytised by the polynuclear cells. Culture was positive for *P. pestis*. At this time fever became more marked and blood culture again became positive. Sulfadiazine therapy was then reinstituted. After five more days of treatment the fever subsided and the blood culture also became negative. Sulfadiazine was then discontinued, though plague bacilli could still be cultured from the cervical and submaxillary buboes. Without any more sulfonamide treatment these gradually shrank in size and were reduced to small fibrotic masses in 10 days' time. The patient was finally discharged in good condition after staying in the hospital for 50 days, having received a total of 146 gm. of sulfadiazine.

This case is interesting in that the plague bacilli persisted in the primary buboes, apparently causing repeated bacteremia and pyemia. It can probably be considered as a case of chronic or subacute plague, the prolongation of illness being probably due to interruption of sulfadiazine treatment.

*Prompt response to sulfadiazine after failure with sulfathiazole.* In the present series, four cases were treated with sulfathiazole and serum at the beginning but were given sulfadiazine later in the course. In one instance the change was made merely because sulfathiazole treatment led to nausea and vomiting. However, the other three patients showed no improvement with sulfathiazole but responded promptly to sulfadiazine. In view of this apparent superiority of sulfadiazine over sulfathiazole, abstracts of their histories are presented.

*Case 2.* H. N. 96. A man aged 36 had acute onset of high fever and a tender bubo in the left inguinal region for 2 days before coming for treatment. He was at first treated with sulfanilamide, 'Sulthia' and 'Thiazon' (local preparations containing sulfathiazole) as well as antiplague serum in insufficient doses. With this scheme of treatment high and irregular fever continued up to the 14th day of disease. Blood culture at this point was positive for *P. pestis*. Sulfathiazole 6 gm. daily were then given for 5 days, but did not lead to a fall of temperature or improvement in the general condition. Blood culture was still positive. It was then decided to change over to sulfadiazine. An initial dose of 3 gm. followed by 1 gm. every 4 hours was used. Temperature gradually came down to the normal level in 3 days' time. With the subsidence of fever, blood culture also became negative. By this time, the bubo had been completely absorbed.

*Case 3.* H. N. 108. A housewife aged 25 was ill for 3 days with a large bubo

in the left inguinal region and was treated with 'Sulthia', sulfathiazole and antiplague serum in another hospital before she was referred to us. She was in deep coma at the time of admission. The tendon reflexes as well as the sphincter controls were all lost. However, she was still able to take fluids and medicine fairly well. Sulfathiazole 6 gm. daily as well as antiplague serum 80 cc. in divided doses was given. Sulfathiazole therapy was continued for a week without effect on the temperature and mental condition. The bubo became greatly shrunk in size but the blood culture was still positive. On the 8th day after admission, sulfadiazine 6 gm. daily was given instead of sulfathiazole. The temperature gradually came down to normal in 2 days but it soon rose again, subsiding finally after 4 more days. By this time the blood culture had become negative and the bubo was no more palpable. With the subsidence of fever, the patient gradually came out from the comatose state. For about a week she was disoriented and extremely excitable, then her mental condition gradually became clearer. At the time of discharge after 24 days of hospitalization, she was considered to be practically normal.

*Case 4.* H. N. 113. A man aged 25 was admitted on the 3rd day of illness. On admission he had high fever and a small bubo in the left inguinal region. Sulfathiazole 4 gm. initially followed by 1 gm. every 4 hours was administered. In addition, 40 cc. antiplague serum intramuscularly were given daily during the first three days. Sulfathiazole treatment was continued for 5 days, but the fever remained at a high level and the bubo became markedly increased in size with extensive local redness and induration. Blood culture, however, which was positive at the time of admission had become negative. From the 6th day of hospitalization, sulfadiazine 6 gm. daily was given instead of sulfathiazole. Temperature came down to normal in 4 days' time. The big bubo went on to suppuration requiring incision and drainage.

*Fatal cases.* There are altogether 5 deaths in the present series, all in patients who came for treatment 3 or more days after onset of illness. Three deaths occurred among patients treated with sulfadiazine alone and two deaths among those receiving combined sulfadiazine and serum therapy. Failure of treatment was due to either an overwhelming infection at the start of treatment or debilitating condition of the patient as shown by the following case abstracts.

*Case 5.* H. N. 137. An apprentice aged 17 was admitted on the 3rd day of illness with a large bubo in the left femoral region. He was said to have massaged the bubo repeatedly since onset. At the time of admission he was critically ill and delirious. He was given sodium sulfadiazine intravenously as well as sulfadiazine tablets by mouth. However, the condition continued to deteriorate. Toward the end, 20 cc. of convalescent serum intravenously and 40 cc. antiplague serum intramuscularly were given without avail. Patient died on the 3rd day after admission, after having received 28 gm. of sulfadiazine.

*Case 6.* H. N. 119. A small, undernourished woman of 57 came to the hospital on the 3rd day of illness. She had a large bubo in the right femoral region and a few discrete, tender glands in the right inguinal region. Sulfadiazine treatment was given after admission. The femoral bubo rapidly increased in

size and the inguinal glands fused to form a big mass. At the same time, local redness and swelling began to appear and rapidly enlarged to involve the whole right thigh and buttocks, extending upward some 6 cm. above Poupart's ligament. Since the appearance of the intense local reaction, she had retention of urine, requiring daily catheterization. On the 11th day of hospitalization, a generalized morbilliform rash developed. At this time, fever had already subsided and both blood and bubo cultures which were positive on admission had also become negative. Sulfadiazine therapy was therefore discontinued. Though the infection appeared to be under control, her general condition did not rally. Extensive bed-sores developed and patient died a few days later.

#### DISCUSSION

The prompt subsidence of fever and disappearance of bacteremia after institution of sulfadiazine therapy in the majority of the cases indicate therapeutic efficacy of this drug in bubonic plague. Of the 16 cases treated with sulfadiazine alone, only three deaths occurred (18.8%). Prior to our study the plague cases in the same hospital receiving other methods of treatment had a fatality rate of 51.4%. The results will stand sulfadiazine in good stead in the treatment of bubonic plague. However, it is futile to compare the case fatality rate of this series with those obtained by other authors using other sulfa-preparations, since the severity of bubonic plague is apt to vary widely. The same comment applies to our results with sulfadiazine in the treatment of bubonic cases during the recent epidemic at Nantien in Western Yunnan (one death in 27 cases or 3.7%) (4).<sup>2</sup> In the present study, the poor results obtained with sulfadiazine plus serum are due to the fact that this combined therapy represents desperate efforts to save the patient's life in the case of severe infection and late in the disease.

The fact that three patients of this series showed no improvement with sulfathiazole but responded promptly to sulfadiazine would seem to indicate the superiority of sulfadiazine. However, the alternative explanation based on selective resistance of the plague bacilli to certain sulfa-derivatives in these particular cases would be equally plausible. The acknowledged fact that sulfadiazine has fewer toxic reactions is a distinct advantage as sulfonamide therapy may have to be continued for a rather long period in some cases.

The present study brings out the fact that to achieve better results, sulfonamide therapy should be instituted early in the course of illness. This is in accord with Pollitzer's results with sulfathiazole in Wenchow where the mortality increased as the interval between onset of disease and start of treatment became longer (9). Similar results have been obtained with antiplague serum by Sokhey (113). Of course patients who can survive the first few days without treatment would have better chances of recovery.

The percentage of positive blood cultures in this series (76%) is quite high. Though some authors assert that bacteremia rarely occurs in bubonic plague,

<sup>2</sup> The authors worked in the epidemic area and helped to compile the statistics.

others claim that initial bacteremia in bubonic cases is constant enough to be of diagnostic value (16, 19). Our results seem to support the latter view. Presence of bacteremia does not necessarily indicate an unfavorable prognosis. It is the severity and persistence of blood infection that should cause concern.

Under sulfonamide treatment the plague bacilli disappear from the blood but, in some cases at least, the organisms still can survive in the bubo for a relatively long time. Considering the pathology of the plague bubo, this is not surprising. As necrosis sometimes occurs in the center of the bubo, sulfonamide concentration there may be very low as compared with the blood level. Therefore, in cases where the temperature has become normal but the bubo still shows sign of activity, one should be on the look-out for possible relapses as seen in one of our patients.

In view of the frequent occurrence of coma, delirium and cardiac failure in severe toxemic cases, good nursing care and supportive measures are just as important as specific therapy. This is especially true in debilitated patients.

#### SUMMARY

In a series of 25 cases of bubonic plague, 16 patients were treated with sulfadiazine alone, 3 with sulfadiazine plus antiplague serum, 4 with sulfathiazole and serum at first but later with sulfadiazine and 2 with sulfathiazole alone. There were five fatalities—3 deaths among patients treated with sulfadiazine and 2 deaths among those treated with sulfadiazine plus serum. While admittedly a small series, the results are sufficiently striking to show the excellent therapeutic value of sulfadiazine in the treatment of bubonic plague. Some interesting findings concerning the importance of early treatment, persistence of plague bacilli in the bubo, occurrence of relapses and instances of prompt response to sulfadiazine after failure with sulfathiazole are presented and discussed.

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# ON THE DDT CONTROL OF SYNOSTERNUS PALLIDUS TASCHENBERG (SIPHONAPTERA, PULICIDAE) IN DAKAR, SENEGAL, FRENCH WEST AFRICA<sup>1</sup>

LEO KARTMAN<sup>2</sup>

## INTRODUCTION

The effectiveness of DDT (2,2-bis(parachlorophenyl)-1,1,1-trichloroethane) against fleas has been well established. Lindquist, *et al.* (1944) showed that powders containing DDT diluted to 4 or 5 per cent with pyrophyllite would control *Ctenocephalides canis* Curtis, *C. felis* Bouché, and *Echidnophaga gallinacea* Westw. on dog hosts. About 10 grams were required to treat a medium sized animal. The dog and cat fleas began to leave the host and drop to the ground within 10 to 15 minutes after treatment. They exhibited spasmodic twitching of the appendages and died in from 3 to 5 hours. Sticktight fleas died on the host.

Davis (1945) found that rat fleas, *Xenopsylla cheopis* Roth., died in 4 hours when placed in a jar with small amounts of DDT. Rats were found to be free of fleas after the application of small amounts of DDT to the fur. Rats were captured before and after buildings were dusted with DDT. The number of fleas per rat in six stores was 13.9 before dusting and 0.6 a month after dusting. Two stores had an index of 0.2 and 0.5 fleas per rat four months after dusting.

Ludwig and Nicholson (1945) had a similar experience during preliminary tests for the control of rat ectoparasites. They dusted rat runs, burrows, holes leading to double floors and walls, etc., with 10% DDT-pyrophyllite dust. This resulted in a 98.3% reduction of *X. cheopis* on rats and an overall reduction of 98.8% of all flea species on rats. This work, and that of Davis, was a part of preliminary investigations to control endemic typhus by the elimination of infected vectors.

Lindquist (1945) reported on DDT-dusted rat runs in buildings and showed that the rat-flea index in untreated areas was 24 while treated areas had an index of 1 flea per rat three months after treatment.

Lewis, *et al.* (1945) in Dakar, French West Africa, reported on the use of DDT against fleas in the native quarter of the city during an epidemic of bubonic plague in 1944.

Kartman (1946) later pointed out that the predominant domestic species of flea in native dwellings of the Dakar region was *Synosternus pallidus* Taschenberg. He suggested, on epidemiologic grounds, that this flea may have possibly acted as a vector of plague to humans in that region, during the outbreak of 1944, since it feeds almost exclusively on man.

The present paper deals with DDT field tests against *S. pallidus* at Dakar

<sup>1</sup> The author wishes to acknowledge the cooperation of personnel of the 19th Malaria Survey Detachment, U. S. Army, Medical Department, during the conduct of this work.

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between May and July of 1944. This work laid the basis for subsequent use of DDT during the plague epidemic, as shown by Lewis and his co-workers (1945).

The primary purpose of these tests was to observe the residual killing action of DDT upon adult *S. pallidus* in native huts and to observe the immediate effect of DDT on adult *S. pallidus* in relation to biting activity. The experiment on

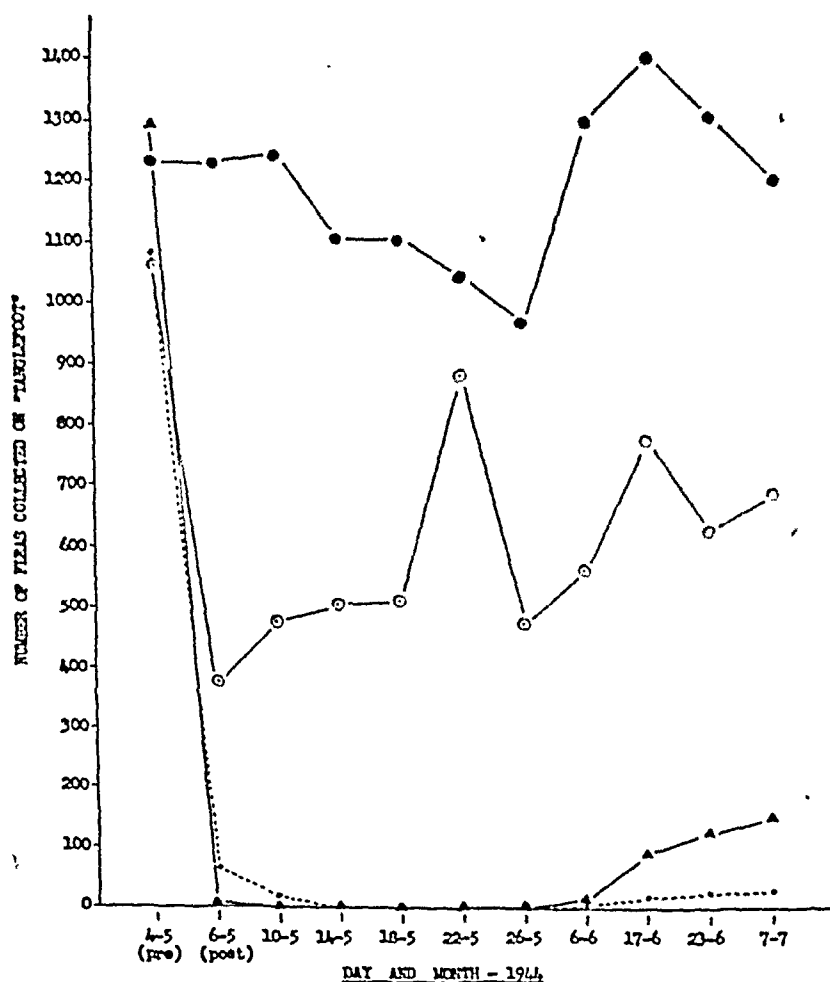


FIG. 1. *SYNOSTERNUS PALLIDUS* POPULATION TRENDS FOLLOWING APPLICATION OF DDT

●—control huts                      ▲—huts air saturated with 5% DDT spray  
○—kerosene sprayed huts          •—huts dusted with 10% DDT-talc

residual kill was made in conjunction with trials on the residual action of DDT against anopheline mosquitoes in a native village near Dakar.

#### METHODS AND MATERIALS

##### *Residual effect*

To test residual effect, twenty five native grass huts (see fig. 2) were selected and divided into the following five groups of five huts each:

NO HUTS	AVERAGE SURFACE AREA OF FLOOR SQ. FT.	MATERIAL USED AND HOW APPLIED
5	108	5% DDT-kerosene, paint sprayed
5	125	5% DDT-kerosene, air saturated
5	121	pure kerosene, air saturated
5	121	10% DDT-talc, dusted
5	117	untreated

In huts which were air saturated, the spray mist settling to the floor constituted the application. In paint sprayed and dusted huts the insecticide was directed to the floor. In all cases, the huts chosen had no straw mats or other floor coverings and all of them were sandy-floored.

The spraying was accomplished by a two-wheeled, pneumatic-tired mobile air unit, working at about 40 pounds pressure, from which two spray units could be operated simultaneously.



FIG. 2. TYPICAL NATIVE GRASS HUTS IN A VILLAGE OF THE DAKAR REGION

These are usually infested with large numbers of *Synosternus pallidus*

(From kodachrome by T/5 Frederick D. Morrison, Medical Department, A.U.S.)

The dusting was done with a rotary hand duster of the type commonly used to cover small areas. No attempt was made to decide on the dosage before treatment. Huts were treated with the object of obtaining adequate coverage and the dosage was estimated after treatment. This procedure was found to be satisfactory under field conditions (especially where the operator was experienced) and later constituted the method employed during the plague outbreak.

A preliminary check on the flea population was instituted during the period directly before the DDT tests. The next check was done about two hours after treatment and succeeding population samples were taken at various periods up to

a maximum of 64 days after the preliminary check. All population counts were accomplished by the utilization of sheets of fly paper ("tanglefoot") in the manner described by Kartman (1946).

### *Biting activity*

To test the immediate effect of a 5% DDT-kerosene solution on the biting activity of adult *S. pallidus*, native grass huts exactly like those indicated above were selected. Test procedure was as follows: The dimensions of the area to be treated were determined so that comparable dosage could be applied under similar conditions in each hut. Flea density in the hut was checked in the manner referred to above and a native Negro with bare feet and legs was then directed to stand in the center of the floor test area while observations were taken on the biting activity<sup>3</sup> of the fleas up to 10 minutes. A measured amount of spray was then applied to the floor. Immediately after spraying, the same native again stood in the center of the test area and the biting activity of adult fleas was observed up to 10 minutes. Fleas were then collected and placed in pill boxes with fresh sand and taken to the laboratory to observe the effects of DDT. The criterion of death was the cessation of all movement when observed under the dissecting microscope.

## DATA AND DISCUSSION

### *Residual effect*

Population checks taken approximately two hours after treatment showed an initial knock-down effect as follows:

TREATMENT	PER CENT REDUCTION OF FLEAS
Paint sprayed with 5% DDT-kerosene	99.7
Air saturated with 5% DDT-kerosene . .	99.5
Dusted with 10% DDT-talc.	95.9
Air saturated with kerosene	75.5
Untreated	0.4

Table 1 summarizes data showing the residual effect of DDT on *S. pallidus*, while figure 1 indicates flea population trends during the same period. It was noted that comparable results were obtained by the two methods of applying 5% DDT-kerosene and by dusting with a 10% DDT-talc mixture. The dust showed a slightly lower initial kill, but its residual effect seemed to be more pronounced at the end of the 64 day period than that of the spray.

Kerosene-sprayed huts showed a poor initial knock-down. The subsequent fluctuation and general rise of the flea population in these huts was quite similar to trends in untreated huts.

<sup>3</sup> It should be noted that the term "biting activity" actually means host seeking activity since observations were made as to whether the fleas would jump and crawl up the host's feet and legs. It is assumed that a flea which jumps upon the host does so for the purpose of feeding, in most cases.

The control huts showed a heavy infestation of fleas throughout the test period with insignificant natural fluctuations despite the fact that large numbers of fleas were collected on fly paper from time to time.

The number of fleas in DDT-treated huts at the end of the test period were negligible when compared to the population of untreated or kerosene-treated huts. At 64 days, DDT-treated huts showed an insignificant rise in the *S. pallidus* population and it may be assumed that this residual action of DDT may have continued for at least several weeks longer. This is indicated by the fact that the population check on the 64th day showed a total of 23 fleas from DDT-dusted huts, 65 fleas from DDT paint sprayed huts, 141 fleas from DDT air saturated huts, 671 fleas from kerosene-treated huts, and 1190 fleas from untreated dwellings.

TABLE 1  
*Residual effect of DDT on Synosternus pallidus in native grass huts*

MATERIAL	HOW APPLIED	NO. HUTS	MG. DDT PER SQ.FT.	FLEA DENSITY† PER HUT											PER CENT REDUCTION WEEKS AFTER TREATMENT		
				2 Days be- fore	Days after treatment												
					4	8	12	16	20	31	42	48	64	1	4	9	
None	Untreated	5	0	245	246	221	220	213	191	255	276	258	238	10	4‡	4	
Kerosene	Air saturated	5*	3 cc.	210	95	168	170	292	154	186	255	208	223	52	46	36	
5% DDT- kerosene	Paint sprayed	5	106	217	0.4	0.2	0	0.2	1	3	13	12	13	99	98	94	
5% DDT- kerosene	Air saturated	5	296	257	0.2	0	0.2	0	0.6	2	16	24	28	100	99	89	
10% DDT-talc	Dusted	5	250	214	0.8	0	0.2	0	0.4	1	2	3	4	100	99	97	

\* Natives dismantled 2 of these huts 8 days after treatment.

† Density = average.

‡ Increase.

The data also indicate that dosages in excess of 100 mg. of DDT per square foot had no appreciable advantage both as regards initial knock-down and residual action against *S. pallidus*. As a matter of fact, one of the huts in the DDT paint sprayed series was treated with 54 mg. of DDT per square foot and showed no strong diminution of residual kill at the termination of observations (see table 2).

### *Biting activity*

Table 3 indicates that about 100 mg. of DDT per square foot, from a 5% DDT-kerosene solution, inhibited the biting activity of adult *S. pallidus* within a very short time after treatment. The fairly uniform results obtained in 8 tests suggest that the dosage of DDT used stopped the activity of the fleas in a minimum of about 10 minutes.

If spraying time were added to observation time, approximately 3 minutes would be added to each figure thus giving 13 minutes as the minimum for cessation of biting activity. The difference is not significant, however.

The 10 minute figure shown in table 3 also applies to the approximate minimum

TABLE 2

*Varying dosage of DDT in individual huts as related to residual kill of Synosternus pallidus*

HUT NO.	MG. DDT PER SQ.FT.	TREATMENT	NUMBER OF FLEAS					PER CENT REDUCTION (WEEKS)	
			2 Days before	Weeks after treatment				2	9
				2	4	6	9		
30	54	5% spray	180	0	5	22	20	100	89
27	99	5% spray	120	0	6	10	14	100	88
29	121	5% spray	72	1	0	15	18	99	75
1	126	5% spray	410	0	4	8	10	100	98
2	130	5% spray	305	0	1	6	3	100	99
16	169	10% dust	187	0	0	16	4	100	98
32	220	5% spray	281	0	0	37	49	100	83
8	231	5% spray	212	0	0	10	8	100	96
18	241	10% dust	306	0	0	1	0	100	100
20	241	10% dust	117	0	0	0	6	100	95
31	259	5% spray	168	0	3	21	38	100	78
19	267	10% dust	214	0	4	2	5	100	98
33	303	5% spray	122	0	8	42	36	100	71
17	334	10% dust	250	0	2	10	8	100	97
6	468	5% spray	502	0	1	14	10	100	98

Untreated Huts

22	none	none	258	278	175	216	222	7*	14
23	none	none	200	106	205	181	140	47	30
24	none	none	179	319	410	289	261	78*	45*
25	none	none	254	235	382	243	267	34	25
39	none	none	236	128	106	365	300	46	27*

\* Increase.

TABLE 3

*Effect on adult Synosternus pallidus biting activity of spraying 5% DDT-kerosene directly on sand-surface of hut floor*

HUT	MG. DDT PER SQ.FT.	NO. FLEAS TAKEN BEFORE SPRAYING	BITING ACTIVITY OF ADULT FLEAS							NO. FLEAS TAKEN FOR OBSERVA- TION 15-20 MINUTES AFTER SPRAYING	ALL FLEAS DEAD HOURS AFTER SPRAYING
			Before spraying (0 to 10 min.)	After spraying (minutes)							
				1	2	3	5	8	10		
1	105	223	+	+	+	+	+	-	-	32	5
2	105	110	+	+	+	+	-	-	-	20	3½
3	102	183	+	+	+	+	-	-	-	46	4
4	105	200	+	+	+	+	+	-	-	17	3
5	104	160	+	+	+	+	+	-	-	25	4
6	105	120	+	+	+	+	-	-	-	39	3½
7	105	205	+	+	+	+	+	+	-	33	3
8	105	181	+	+	+	+	+	-	-	51	4

+ Indicates that fleas were observed to jump and crawl on feet and legs.

- Indicates that fleas were not observed to jump upon feet or legs.

time that *S. pallidus* adults were first seen to exhibit typical DDT toxicity.<sup>4</sup> This consisted mainly of spasmodic twitching of the appendages. The onset of toxic symptoms and the inhibition of host-seeking were of simultaneous occurrence. Actual death of the fleas varied from 1 to 5 hours for separate individuals. The death of all fleas in each of the tests gave a variation of from 3 to 5 hours with an average of  $3\frac{3}{4}$  hours. This is in accord with the observations of Lindquist, *c' al.* (1944) and Davis (1945).

It is also interesting to note that larvae of *S. pallidus* were seen in great numbers several minutes after the floors of huts were sprayed with the DDT-kerosene solution, whereas they were not in evidence preceding treatment. Close observation showed these larvae to be twitching spasmodically and specimens taken to the laboratory died in from 4 to 7 hours.

#### SUMMARY AND CONCLUSIONS

In view of the possible importance of *Synosternus pallidus* Taschenberg as a vector of plague to humans, field experiments are described which tested the residual action of DDT upon adults of this flea species in native huts of the Dakar region of French West Africa.

Data are also given on the immediate effect of DDT in relation to the biting activity of *S. pallidus*.

A 5% DDT-kerosene solution, when paint sprayed directly upon the floor or air saturated, gave an approximate 100% initial kill of adult *S. pallidus* and a residual kill which extended to 64 days or more.

A 10% DDT-talc dust, when applied to the floors of native huts, gave initial and residual kill of *S. pallidus* adults comparable to that of a 5% DDT-kerosene spray. The DDT dust seemed to produce a slightly lower initial kill and a slightly longer residual action than the spray, although the difference was not significant.

Dosages in excess of 100 mg. of DDT per square foot showed no appreciable advantage both as regards initial knock-down and residual action against *S. pallidus*.

The air saturation of native huts with a pure kerosene spray gave a poor initial kill and no residual effect of any significance on *S. pallidus*.

The biting activity (host seeking) of adult *S. pallidus* was inhibited approximately 10 minutes after the floor of a native hut was sprayed with a 5% DDT-kerosene solution at the rate of about 100 mg. of DDT per square foot.

Adult *S. pallidus* exhibited typical DDT-toxicity in a minimum of 10 minutes after floors were treated. These fleas died in from 3 to 5 hours after contact with DDT.

The initial and residual kill by DDT of *S. pallidus*, on a large scale under practical field conditions, suggests that this may be an effective addition to established

<sup>4</sup> The question may arise as to the effect of pure kerosene. In this connection, observations showed that kerosene, although it killed many fleas, did not produce toxic symptoms which inhibited biting activity.



procedure in the prophylaxis and control of flea-borne disease. This would obtain especially in conditions where the flea species is highly domestic, feeds on humans, and is known to be a vector of plague or other diseases from man to man.

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# A LABORATORY INFECTION OF THE RAT WITH FILARIAL WORMS<sup>1</sup>

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A laboratory animal which can be infected with definite numbers of some type of filarial worm has long been needed for the study of filariasis. The experiments reported here show that the white rat and the cotton rat can be infected in the laboratory under repeatable conditions with *Litomosoides carinii*, a filarial worm of the cotton rat. The vector apparently being one of several arthropods which have become established in the experimental units. Experiments now in progress should make possible the isolation of this vector and thus demonstrate the actuality of transfer by a particular species. The advantage of such a demonstration, beyond its immediate value in providing an infectible laboratory animal, has been well stated by Hinman (1935) as follows:

"The maturation of the larvae within the insect host and its migration to the proboscis has always been assumed to be *prima facie* evidence of ability to transmit the given filarial infection. However, it is entirely possible that many blood-sucking insects, discovered to be susceptible to experimental infection and even found naturally infected, are unable to successfully transmit the parasite. Practically all of our knowledge of transmission of filarial organisms rests upon such circumstantial evidence, which may be quite fallacious."

Several authors have reported experimental infections with *Dirofilaria immitis* in dogs, but the results have not been sufficiently consistent to result in the establishment of laboratory strains of the parasite which can be propagated by serial transfers. The experiments of Grassi and Noe (1900) have been criticized on the ground that their experimental dogs were raised in an endemic area and might have had a natural infection. Bancroft (1904) infected several dogs under more adequately controlled conditions, but it is not clear how many failures he may have had. Fulleborn (1929) infected 5 dogs which apparently represented all that he tried to infect. On the other hand, Hinman (1935) was unable to infect any of 3 dogs by repeatedly feeding *Aedes aegypti* on them, even though other mosquitoes of the same lots developed infective larvae in their heads. Apparently one of the reasons why *Dirofilaria immitis* has not become a laboratory infection is the difficulty of producing the infection. Perhaps this difficulty would have been solved if the dog were not such a relatively expensive animal to raise and maintain in the laboratory. Moreover, the infection in the dog is not entirely satisfactory for many types of experiments because the position of the worms in the heart differs so greatly from that of the human filariae.

Highby (1943) reported the experimental production of light infections in

<sup>1</sup> Read at the 41st meeting of the American Society of Tropical Medicine at Cincinnati, Ohio, November 15, 1945.

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domestic rabbits of *Dirofilaria scapiceps* from the snowshoe hare. Although this species may be useful as experimental material, there is no indication that a laboratory strain could be established with any degree of facility.

Lizards have been experimentally infected with a filaria (Pandit, Pandit and Iyer, 1929; Menon, Ramamurti and Rao, 1944) which is pathogenetically more similar to the human filariae. It is not known, however, that these lizards could be raised in the laboratory or that they would thrive away from their native habitat.

*Litomosoides carinii*, a parasite of the cotton rat, has recently come into extensive use in the study of filariasis especially in connection with experiments on chemotherapy. It has been possible to obtain large numbers of naturally infected, wild-caught cotton rats, but for many types of experiments, infections of known age and approximately equal intensity are desirable. In connection with his original description of this worm, Chandler (1931) mentioned that a white rat kept in the same room with infected cotton rats had become infected. The present studies originated as an attempt to duplicate the above transfer under somewhat more precisely controlled conditions. When this transfer had been successfully accomplished, the experiments were expanded to repeat the transfer to cotton rats.

#### METHODS OF STUDY

Transmission of the infection has been successfully accomplished at all trials regardless of the details of the methods. In the first experiment, a pair of cotton rats, *Sigmodon hispidus texianus*, infected with *Litomosoides carinii* and a laboratory-raised white rat were placed in a double cage on opposite sides of a wire partition which extended through a wooden nesting box. Several grass nests of cotton rats were placed in the box immediately after they were brought from the field. A wire floor allowed the droppings to fall into a tray, but neither tray nor cage were cleaned for two months. The whole unit was protected from crawling insects by being placed in a large oil pan and was located in a screened building. A few mosquitoes, *Aedes sollicitans*, did, however, occasionally gain temporary access to the animals when attendants entered the room. The building was located on Galveston Island 10 miles from the nearest habitat of the cotton rat, which is on the mainland across 2 miles of open water. The male cotton rat was removed after 3 weeks, and 4 young were born on the 34th day of the experiment, thus adding 4 more test animals to the experiment.

In the second and third experiments an infected cotton rat and her newborn young were placed immediately after their birth in each of two galvanized iron tanks. These tanks measured 18 x 24 inches, and 18 inches deep, and were set on legs standing in oil cups to prevent the entrance of crawling arthropods. Moreover, the tops of the tanks were provided with an oil-filled moat and were tightly screened with 18 mesh wire. In the bottom of the tank was placed two inches of washed sand on top of one inch of washed gravel and this sub-stratum kept slightly moist. On the sand there was a 2-inch layer of dried grass of the type used by cotton rats for nesting material. Two complete cotton rat nests

freshly gathered in the field were also placed in each tank at the time the rats were introduced. These first rats were not freed of ectoparasites. Washed carrots and baked dog chow were provided in non-spillable feeders. Every few days the feeders were removed for thorough cleaning and any arthropods found in their wire-covered trash pans were returned to the appropriate tanks. The tanks were kept in a screened building in Galveston, and throughout the experiments all conditions were maintained as constant as possible.

As will be shown below, numerous control animals were housed in cages in the rooms with the experimental units and 24 were killed at various intervals, paralleling the autopsies of experimental animals. The negative results show that no transmission occurred outside of the experimental cages and tanks, even among litters born to infected mothers.

#### RESULTS OF THE EXPERIMENTS

The results of the experiments are summarized in table 1. In the first experiment the white rat was killed after having been housed with the infected cotton rat for 57 days, and was found to harbor 3 immature worms. These worms and all those recovered from the cotton rats were positively identified as *Litomosoides carinii*. All 4 cotton rats born in this cage on the 34th day of the experiment were found to be infected with mature worms when autopsied at ages varying from 107 to 147 days.

In the second experiment an infected cotton rat and her 4 newborn young were placed in the tank described above, along with freshly gathered cotton rat nests. One young rat died 42 days later and was found to harbor 3 immature worms. The other three young rats were removed from the tank on the 54th day and were killed at ages varying from 66 to 93 days. All were harboring mature worms. To continue this experiment, the original mother was removed on the 54th day and another cotton rat with 2 newborn young (all were ectoparasite free) were substituted without other changes of conditions. In other words, the old nests remained, but there was no addition of new nests or arthropods. After 41 and 42 more days, respectively, the young rats were removed and killed. One harbored 2 immature worms and the other none. The mother was removed 45 days after her entry into the tank and was found to harbor 24 distinctly immature worms, some as small as 1.5 mm. in length as compared with worms 22 mm. in length found in young rats 42 days of age. As she had been caged for some time since capture, these small worms must have been acquired in this tank and the very smallest only shortly before autopsy. In other words, the vectors were apparently still present in the tank approximately 3 months after the only arthropod-containing material had been added. This rat represents an uncontrolled experiment as compared with the young rats, of course, but the information does seem to add something of value to the other more positive evidence. She also harbored over 100 larger worms, some of which were evidently acquired in the tank while some fully grown worms apparently represented the original infection acquired before capture.

The third experiment was similar to the first part of the second one. Four

newborn cotton rats with their infected mother and her ectoparasites were put into a tank with newly gathered nests. The young rats were killed at periods varying from 42 to 83 days and all were found to be harboring either mature or immature worms.

TABLE 1

*Showing the results of housing together infected and uninfected animals in the presence of freshly gathered cotton rat nests*

At intervals throughout the period covered by these autopsies, 24 control rats which had been housed in cages in the same rooms were autopsied and all found negative.

RAT NUMBER	DATE OF ENTRY INTO UNIT	DAYS FROM ENTRY TO REMOVAL	DAYS FROM ENTRY TO AUTOPSY	DAYS FROM ADDITION OF ARTHROPODS TO REMOVAL	NUMBER OF WORMS HARBORED
Experiment 1. (Double Cage)					
W-1*	4/10	57	57	57	3
145	5/14	101	107	135	5
146	5/14	101	139	135	3
147	5/14	101	144	135	6
148	5/14	101	147	135	5
Experiment 2. (Iron Tank)					
200	7/30	42	42	42	3
198	7/30	54	66	54	4
199	7/30	54	68	54	10
197	7/30	54	80	54	8
201	7/30	54	93	54	5
221	9/22	41	41	95	2
222	9/22	42	42	96	0
49	9/22	45	45	99	24†
Experiment 3. (Iron Tank)					
215	8/9	42	42	42	8
212	8/9	47	49	47	11
210	8/9	47	58	47	11
211	8/9	47	65	47	9
213	8/9	47	75	47	11
214	8/9	47	83	47	2

\* W-1 was a white rat, the others were all cotton rats and with the exception of 49 were born on the day of entry into the units.

† 49 was the mother of 221 and 222 and these 24 worms represent only those which were distinctly immature.

Summarizing the results of these experiments: success has been attained in transmitting the infection in each of three trials of housing together infected and uninfected rats in experimental units having a moist substratum on which freshly gathered cotton rat nests had been placed. In the three trials a total of 15 out of 16 cotton rats became infected, as well as the only white rat tried, while 24 parallel control cotton rats and 4 control white rats were uninfected.

## EVIDENCE REGARDING THE VECTOR

During the past year 143 cotton rats have been trapped on the mainland part of Galveston County, Texas. Of these, 92 weighed over 100 gms. when caught and 65 of these larger rats, i.e. 85 per cent, were found to be harboring *L. carinii*. The percentage of infection among smaller rats was slightly less, increasing with apparent age until they had reached the weight of 100 grams. At all seasons of the year enough immature worms have been found in newly caught rats to show that while there may be a seasonal variation in the rate of infection, some infection apparently does occur throughout the year.

With such a high prevalence of infection, a vector which is abundant in the habitat of the rats seemed to be indicated. Dr. Paul D. Harwood<sup>2</sup> had failed to infect the seven species of mosquitoes found in the habitat of these rats in the adjoining Houston area. Extensive ecological studies made during the early phases of the program of which the present study is a part, also indicated that mosquitoes were not likely to be the vectors. The experiments detailed above have definitely eliminated mosquitoes as essential vectors. Other biting diptera have been virtually eliminated. *Phlebotomus* is not found in southeast Texas and *Culicoides* or other midges have not been captured in careful searches of the environs of the experimental units. The search therefore seemed to have narrowed down to the ectoparasites of the cotton rat, and almost certainly one of these was the actual vector in the experiments reported here since no other biting arthropods have been found in the tanks.

Out of 112 cotton rats caught in the area under conditions which made a determination of their ectoparasites possible, 63, or 56 per cent, were found to harbor fleas, *Rhopalopsyllus gwyni*. The number of fleas per rat varied from 1 to about 15, 4 or 5 being the commonest number. Ticks were found on 13 of these 112 rats, but they were eliminated from consideration as the essential vector by their absence from the experimental units. Mites were found on 66, i.e. 59 per cent, of these rats in numbers varying from 1 to 20, the median number per rat being somewhere between 5 and 10. Part of these mites were mounted for taxonomic studies, others have been dissected (after positive identification) in a search for larval worms, while a third group has been used in attempts to establish laboratory colonies. Of those identified, four specimens from as many rats were *Liponyssus bacoti* while the rest belonged to a new species of *Atricholaelaps* which is being described by Dr. Russell Strandtmann of this department. Since Dr. Strandtmann has been working with *Liponyssus* for some time, his help has been valuable to us in our attempts to separate this species from the more common new one while the specimens are still alive and unmounted. As a result we are fairly confident that all of those used for cultures were *Atricholaelaps*, even though they were not mounted for careful study. In any case, it is certain that *Liponyssus* is only rarely obtained from these rats when they are brought immediately from the field to the laboratory in wooden box traps of a type recently described by one of us (Scott, 1945).

<sup>2</sup> Personal communication.

In the tanks of experiments 2 and 3, *Atricholaelaps* has become well established, hundreds and occasionally thousands of specimens being found in the trash trays of the feeders. Only rarely, however, have one or two *Liponyssus* been seen in these trays, but once during the 5th week of experiment 2, 6 or 8 specimens were found, of which several were mounted for positive identification. Since the first rats introduced into each unit at the time the nests were added were not freed from their ectoparasites, a few fleas were introduced. They have multiplied only slightly, most rats having from one or two to fifteen on them when removed from the tanks. None have been found in any material taken from the tanks. Some of these fleas were returned to the tanks each time while the rest were dissected after all were identified as *Rhopalopsyllus gwyni*.

On the grounds of abundance, then, both in the field and in the experimental units the evidence points to either *Atricholaelaps* or *Rhopalopsyllus* as the vectors. The results of dissections do not support this evidence, however, since nearly 100 fleas and over 500 *Atricholaelaps* taken from infected rats or from experimental units containing infected rats have been dissected, but no developing forms have been seen. Parenthetically it should be noted that a species of mite of the family *Chyletidae* was found in the cage of the first experiment, but there is no evidence that they are parasitic, the results of over 300 dissections were negative and they were not present in the second and third experiments.

Since the first few specimens of *Liponyssus bacoti* from the rats and from the tanks were needed for taxonomic purposes, too few have been dissected to warrant conclusions from the negative results obtained. It may be significant that the only living microfilariae found in any dissection were from one of these mites. The absence of infections in the other available species would seem to incriminate *Liponyssus bacoti* by a process of elimination. Its scarcity in the experimental units would, however, argue against its having been able to transmit the infection so regularly. Moreover, the fact that it has not been found as yet in a search through several cotton rat nests and the rarity of its occurrence on wild-caught rats would point in the same direction. On the other hand, we have observed that this species becomes much more distended after feeding than *Atricholaelaps*, indicating that it may be a much more intermittent feeder and may tend to drop off after feeding. If it also has some peculiar burrowing or hiding habit which we have not discovered, it may be more abundant than we think. It might also be possible that it is a very efficient vector and that the infection is fatal to it. If, under such conditions, the female had time before death to lay enough eggs to just maintain the population, the observed facts might well be explainable.

#### SUMMARY

Successful transmission of infections of *Litomosoides carinii*, a filarial parasite of the cotton rat *Sigmodon hispidus texianus*, has been accomplished in all of three trials made by housing together infected and uninfected rats in a moist environment into which freshly gathered grass nests of cotton rats had been introduced. Altogether 15 out of 16 cotton rats became infected as well as the only white rat tried, while 24 control cotton rats and 4 control white rats remained

uninfected. The only biting arthropods found in the experimental units were fleas, *Rhopalopsyllus gwyni*, and two species of mites, viz. a new species of *Atricholaelaps* and *Liponyssus bacoti*. On the grounds of abundance both in the units and on wild-caught rats either the fleas or the former species of mites seemed indicated as the vector, but 100 and 500 dissections respectively revealed no developing larvae. *L. bacoti* was rare both on wild rats and in the units and too few were dissected for conclusions, but this species seems to be incriminated by the elimination of other possibilities.

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## BOOK REVIEWS

*The Principles and Practice of Tropical Medicine*, by L(ionel) Everard Napier, C.O.I.E., F.R.C.P. (London), formerly Director and Professor of Tropical Medicine, Calcutta School of Tropical Medicine; formerly Consultant to the Secretary of War and Visiting Lecturer on Tropical Medicine, Army Medical School; Visiting Lecturer on Tropical Medicine, Harvard Medical School; formerly Visiting Professor of Tropical Medicine, Tulane University and Visiting Professor of Medicine, New York University. XVI + 917 pages, with 195 text figures and colorplates A-D. The Macmillan Co., New York, 1946.

This impressive volume is the record of a long period of experience and observation on the part of an able worker in the field of tropical medicine. For the most part it is based on data collected in India where the author spent many years in fruitful endeavor. The first 522 pages of the text were published without index in 1943; the remainder, including the index, while the author was visiting professor in the United States. In undertaking an evaluation of this contribution the reviewer has attempted to be sympathetic with the circumstances of publication, but at the same time he has been obliged to judge its merits objectively.

Following a preface and general introduction, in which the point of view of medical practice in the Tropics is presented, there are three chapters dealing primarily with tropical environment, their effect on the human constitution and on disease. In considering the centers for special knowledge in tropical disease at the beginning of the present century (p. 2) Dr. Napier was apparently unaware that at that time Philadelphia and New Orleans were as important as London, Hamburg and Baltimore. In defining tropical medicine and its scope (p. 3) the author gives the impression of being uncertain as to what this concept comprehends. In considering "Environment and Distribution of Disease" (pp. 4-15), "Measures for Mitigating the Effects of Tropical Climate" (pp. 16-32) and "Diseases Due to the Direct Effects of a Tropical Climate" (pp. 33-51) the discussion is well documented and the recommendations are well presented and fundamentally sound, with illustrations drawn primarily from observations in India. A serious omission is the failure to include the comprehensive study by A. Grenfell Price on "White Settlers in the Tropics" (1939), which provides valuable data on a variety of tropical environments.

The first specific disease to be considered in detail is "Malaria" (pp. 54-123). This is appropriate since malaria is the most important and most widely disseminated disease in warm climates. Most of the coverage is excellent but several comments are appropriate. The map showing world distribution of the disease (p. 55) is too small for effectiveness and is not up-to-date. In listing the *important anopheline vectors* (p. 66) the reader's attention is called to the inclusion of *A. crucians*, which has never been an important transmitter, while *A. gambiae* has not been present in Brazil since 1940. In this latter connection the introduction of *gambiae* "into South America, where it has caused a most disastrous increase in malaria," possibly suggests that this vector became widely established, whereas it was never encountered except in northeastern Brazil. Malaria pigment should no longer be designated as "haemozoin" (p. 61), since it has been proven to be hematin. The exo-erythrocytic stage of the malaria parasites is suggested in the diagram on p. 62 but the reviewer has found no mention of it in the text. The fundamental implications of this phase of the infection have been particularly appreciated following the clinical studies of Brigadier Fairley (1945) on incubation, latency, relapse and therapeutics in vivax and falciparum malaria.

Possibly the most serious imbalance in material presented on malaria is the small amount of space given to atabrine in the treatment of clinical cases and in suppressive administration. Even with the realization that the results of Brigadier Fairley's work may not have been available for publication at the time this part of Doctor Napier's volume went to press, there were earlier publications from Malaya, India and the Panama Canal Zone to

commend its routine use even when quinine is available. Although a brief footnote mentions DDT, this recent control armamentarium is not included in the subject index. The latest references on malaria bear the date 1941!

In the chapters beginning with "kala-azar" (p. 136) "Prevention" is usually taken up immediately after "Diagnosis" and before "Treatment." The reviewer has not been able to fathom the logic of this particular sequence.

"Kala-azar (Visceral Leishmaniasis)" is a disease on which Doctor Napier is an outstanding authority. Hence it is expected that he should give more space to it than might otherwise be justified. In general, there is splendid presentation of the epidemiology and clinical aspects of kala-azar. Incidentally, mention is omitted of the endemicity of kala-azar in Szechuan and Sikong provinces in West China. (Yunnan Province is referred to as "Yunan," Hopeh as "Chih-li" and Fengtien as "Fiengtien." The incidence and geographical distribution of the disease in South America are given too casual mention. In the consideration of specific therapy in kala-azar the reviewer is disappointed in the brief references to antimonials other than the tartrates, neostibosan and urea stibamine, while undue space has been devoted to the aromatic diamidines.

The chapter on Oriental sore (cutaneous leishmaniasis), presenting another disease with which Doctor Napier has had intimate experience, is well-balanced and does full justice to the subject. On the other hand Muco-cutaneous Leishmaniasis, which is headed "South American Muco-cutaneous Leishmaniasis," but is described as from South and Central America, is incomplete and not up-to-date. The only references to infection with *Leishmania brasiliensis* post 1926 are three which are cited from Tropical Diseases Bulletin.

The reviewer had had no personal experience with "African Trypanosomiasis" but is favorably impressed with the presentation of this dual disease (due to the closely related agents *Trypanosoma gambiense* and *T. rhodesiense*). Doctor Napier rightly emphasizes the importance of symptomless carriers of *T. gambiense*, and the diagnosis of chronic cases by spinal fluid examination, but he fails to mention that the aromatic diamidines are potentially valuable in the acute stage of the disease when tryparsamide may not be tolerated or when the strain of the organism is drug-fast to arsenicals or Bayer 205.

In considering "Chagas' Disease (South American Trypanosomiasis)" there is considerable evidence of inadequate coverage and failure to check text and illustration. Although the text (p. 219) mentions most of the countries in which the disease has been discovered in man, only three (Brazil, Argentina and Venezuela) are shown on the map. Possibly a more serious omission is the failure to give a clear picture of the sequence of development of the lesions in this disease from the primary site through the blood stream to the reticulo-endothelial system, myocardium, adrenal cortex and central nervous system. The list of four references is totally inadequate.

The author appears to be uncertain of terminology in his reference to the etiological agents of "The Relapsing Fevers." In fig. 52 (p. 225) it is *Treponema recurrentis* for the louse-transmitted types; in the text (p. 226) it is *Spirochaeta recurrentis*. Only one line (p. 232) is given to DDT in the prevention of louse-borne infection. Novarsenobillon is recommended for specific treatment, and penicillin is regarded as impractical because the computed dosage for man, based on small animal experimentation, is excessive. Clinical studies have shown that a much smaller dosage is effective. It would have been helpful in discussing the tick-transmitted strains of relapsing fever spirochetes to mention the many species of *Ornithodoros* other than *moubata* and *erraticus* which are involved as vectors of this spirochete. *Rhipicephalus sanguineus* is stated by the author to transmit this disease from dog to man.

No consideration has been given to the preventive aspects of rat-bite fever. In the chapter on "Leptospirosis" China and the Dutch East Indies are not mentioned, although they are important endemic countries. The only mention of *Leptospira canicola* is that this "strain is common in the United States" (which is contrary to reported evidence). Penicillin is not included in the therapeutics of this disease.

Only thirty pages are devoted to "The Typhus Fevers." In the classification of the

etiological agents Doctor Napier proposes (footnote, p. 260) a new superfamily, Rickettsioidae, to include the family Rickettsiaceae for the pathogenic species and the family Wolbachiaceae (n.n.) for the non-pathogenic ones. It must be pointed out that the International Rules of Botanical Nomenclature which are involved do not recognize superfamilies and even if they did the zoological suffix *oidae* would not be used; moreover, it would be confused with *oidae* which is the botanical subfamily suffix. Bolivia is not included in the countries where louse-borne typhus is known to have occurred in recent years (p. 261).

The reviewer is not able to understand just how the feces of an infected louse serve as "the sole source of infection of the next generation of lice" (p. 262). On the following page, under "Pathology," it would have been helpful if the arteriolar lesion had been designated as "Fränkel's nodule." Exception is taken to the statement on page 268 that the causative organism of murine typhus "is usually known as *Rickettsia muricola*" (i.e., it is usually referred to in the literature as *R. mooseri* or *R. prowazeki mooseri*). Again, tabardillo is not the appropriate term for endemic typhus of "the southeastern states of the U. S. A." (p. 268). Attention is called to the fact that "tsutsugamushi" means "mite" and should not be used to designate mite-transmitted rickettsiosis without the accompanying word "disease" or "typhus." Finally, the section on the typhus group of fevers suffers because it was printed in 1943 before differential complement fixation tests became established and before immune serum and para-aminobenzoic acid were accepted as therapeutic agents for these diseases in man.

In the brief chapter on Oroya Fever Bolivia is erroneously reported as a focus (p. 289). No reference is made to the important epidemiologic studies of Hertig (1942).

The chapter on Yellow Fever is well balanced and well presented and has been brought up through the year 1944 by an addendum. In the dengue-sandfly fever group it is stated that "*Aedes* alone transmits" dengue (p. 314). *Armigeres* is a known transmitter. The presentation of sandfly fever is rather unsatisfactory; it suffers particularly from failure to include the valuable data of Sabin, Philip and Paul (1944).

In the chapter on Plague the map (p. 323) correctly shows an active focus of the disease for northeastern Brazil, although there is no mention of this area in the text. Tularemia is stated (p. 346) to be caused by *Brucella tularensis* (SIC). In the undulant group of fevers the valuable contributions of Ruiz Castañeda (1941 *et seq.*) have been overlooked.

In reading the introduction to diarrheas and dysenteries ("The Intestinal Fluxes," pp. 368-369) the reviewer can not subscribe to the following statements:

- (1) "Amoebic dysentery. Primarily a dysenteric condition;"
- (2) "Flagellate dysentery. A diarrhoeal and occasionally dysenteric condition."
- (3) "Ciliate dysentery. A rare but serious condition."

As the average physician knows, amoebic dysentery constitutes only a small percentage of cases harboring *E. histolytica* and is not a major clinical manifestation in most endemic areas. Moreover, there is no proof that *Giardia lamblia*, *Trichomonas hominis* or *Chilomastix mesnili* initiates dysentery. *Balantidium coli* is not a rare cause of dysentery in Venezuela and certain other countries of northern South America.

The chapter on cholera is excellent and leaves little to be desired.

Although Doctor Napier has followed tradition in referring to *Shigella* infection as "Bacillary Dysentery," he admits that the disease manifestations produced by species of *Shigella* "vary from a mild diarrhoea, in which the patient is scarcely inconvenienced at all, to a very severe toxæmic attack which simulates a severe attack of cholera; in neither of these extreme cases does the true dysentery picture appear" (pp. 405-406). Yet he avoids the newer designation "shigellosis," because "it is at present a little confusing for clinicians." No mention is made of the valuable epidemiological studies of Hardy and Watt (1942 *et seq.*) on acute diarrheas, or of the particular usefulness of autogenous vaccines in the treatment of chronic *Shigella* infections.

The subject of amebiasis is presented under two chapter headings, "Amoebic Dysentery" and "Amoebic Hepatitis and Liver Abscess." Although the author refers (p. 425) to the

modern view that amebic dysentery is only one aspect of amebiasis of the bowel, he prefers to adhere to the older concept by referring to it as "amebic dysentery," and thus is forced to resort to elaborate, albeit unsatisfactory explanations for the large proportion of cases of amebic colitis lacking a dysenteric syndrome. His statement (p. 426) that "amebic dysentery is a rare incident" in the temperate zone is a particularly difficult one to interpret, since the reader is not certain if the author means frank dysentery (*sensu stricto*) or amebic colitis. However, true dysenteric manifestations in amebiasis are not rare in patients who have never been outside the United States, as the records of the Mayo Clinic and many other medical institutions in the United States testify. Possibly Doctor Napier has drawn his inferences from a comparison of Indian and English data. Moreover, the older idea that amebiasis "is not common amongst children" (p. 427) is no longer tenable.

In discussing laboratory diagnosis of amebiasis (pp. 436-438) no mention is made of the usefulness of D'Antoni's iodine stain or of the zinc sulphate centrifugal flotation technique for concentrating amebic cysts from feces. The reviewer can not accept the statement that the presence of Charcot-Leyden crystals is significant. In the majority of stools of persons suffering from amebic colitis these bodies can not be demonstrated. The excellent diagnostic figures of amebic trophozoites and cysts (Plates XI and XII), which have been borrowed from Dr. John F. Kessel, are unlabelled.

In the treatment of acute or chronic "amebic dysentery" Doctor Napier places his faith on emetine, bismuth and carbarsone, with emetine bismuth iodide as an alternative and chiniofon, vioform and diodoquin incidentally mentioned under treatment of the chronic infection. For a comprehensive evaluation of antiamebic therapy the reader is referred to the excellent paper by Professor A. R. D. Adams, "Amebiasis with special reference to treatment" (Trans. R. Soc. Trop. Med. and Hyg., 38(4), 237-244, 1945). In the volume under review there appear to be no recommendations for follow-up stool examinations.

As a whole the consideration of amebiasis of the liver is sound but the statement (p. 450) that "there appears to be no danger of liver abscess developing in the intestinal amebic infections that occur in temperate climates" is certainly not borne out by the experience of the Mayo Clinic, the Duke Hospital and the numerous clinical studies on amebiasis in New Orleans. This chapter lacks a bibliography.

Sprue is defined as "a diarrhoeal condition of uncertain aetiology" and is separated by nearly 300 pages from the deficiency diseases.

The chapter on Leprosy (pp. 481-522) written by Dr. John Lowe, is a sound and distinguished contribution to medical literature.

Relatively brief but adequate space is devoted to Yaws, Pinta and Bejel (pp. 523-542). Under "Tropical Skin Conditions" the commonly used designation "cutaneous diphtheria" is not mentioned in the discussion of ulceration caused by the Klebs-Loeffler bacillus (pp. 551-554).

The reviewer is disappointed in the limited scope given to mycotic infections. Except for the common dermatophytes no consideration is given to this prevalent and important group of disease-producing agents in the Tropics. The systemic mycoses are not presented.

The portion of the book allotted to "Helminthic Infections" (pp. 586-746) is adequate and in some chapters the presentation is good. The best and most useful chapters on helminths are the two on "Filariasis" and "Dracontiasis," fields in which the author has had years of experience. Several of the other chapters bear witness to inadequate personal contact with the infection or acquaintance with the work of students of the subject. The reference citations are not comprehensive. It is unfortunate that inconsistencies occur as a result of the author's selection of this material from two groups of workers who use different terminology, viz., "*Trichocephalus trichiurus*" vs. "*Trichuris trichiura*" (Table XV, p. 588 and Tables XVI, XVII, pp. 591, 592, *et seq.*); "egg" and "ova" (text, p. 596 and fig. 143, p. 597). The use of the common name "threadworm" (p. 600) for *Enterobius vermicularis* is unusual; this is more frequently reserved for *Strongyloides*. However, this merely illustrates how unsatisfactory nonscientific names are for parasitic agents of disease. In

The taxonomic table, "Diagnosis and Treatment of Intestinal Helminths" (Table XVIII, p. 544), the common tropical hookworm, *Necator americanus*, is not included.

The reviewer does not consider himself qualified to comment on the group of diseases presented under "Nutrition and Nutritional Disorders in the Tropics" (pp. 745-835).

Although the chapter on "Snakes and Snake Bite" (pp. 836-859) lacks sufficient details for the foreign-born outside India, nevertheless the general presentation is informative. The first chapters of p. 864-867 on "Rabies," and "A Note on Myiasis and Scarabiasis" are brief and are not connected with other groups of subject matter.

In reading the volume for content, method of presentation and balance rather than presiding at for typographical errors the reviewer has probably missed some of the most obvious lapses in orthography. A few which have been discovered include: p. 3, last line, "Atrax" for "Acanax;" p. 287, footnote, "etain" for "strain;" p. 289, next to last paragraph, "pachii" for "pachii;" p. 323, 2nd paragraph, 2nd line, "Yunan" for "Yunnan;" p. 323, Fig. 20B, "silvatic" (of Portuguese derivation) for "silvatic" (of Latin origin); p. 426, 2nd paragraph, first line, "Loch" for "Lösch;" p. 427, 2nd paragraph, "butschlii" for "butschlii;" p. 451, 2nd line of text, "infest" for "infect;" and p. 589, Table XV, "Cestoiden" for "Cestoides" and "Cyclophyllidea" for "Cyclophyllidae."

Brief comment should be made on the illustrations. There are 195 text figures, including maps, schematic diagrams, temperature charts, graphs, line and stipple drawings, and photographs. World maps showing distribution of disease frequently suffer from too great reduction. Some of the line and stipple illustrations are diagrammatically effective but occasionally they are not accurate. Those on helminth eggs could have been reduced to advantage. Several of the life cycle and epidemiological diagrams in the section on Helminths would have been more effective if the blocks had been left unshaded; the shading produces a disturbing optical impression (viz., Fig. 143 p. 597) which considerably reduces the value of the illustration. The photographs are on the whole too dark. This comment applies particularly to the ones on Bancroft's filariasis (Figs. 166-175). Of the 4 colored plates listed (only 3 have been found by the reviewer) the first (Frontispiece A) detracts from the appearance and value of the book because of the inartistic, unlikelike and frequently inaccurate individual illustrations. In Frontispiece B the illustrations of *Treponema recurrentis* (H) and *Leptospira icterohemorrhagiae* (J) are misleading with respect to relative size.

The format of the book is fairly good, but too much space is employed in the large-sized, widely separated outlines of material presented at the beginning of several of the chapters and space is frequently wasted where a short reference list occupies a full page. Some of the pages have blurred print. The subject and author indexes are fairly complete but not exhaustive. The reviewer's copy is poorly bound, with the cloth cover of the board unglued on arrival and mottling which was apparently caused by dampness in transit through the mail. These qualities hardly recommend the volume for use in the moist Tropics.

If the reader has had the patience to follow through to the end of this review, he will note that there is much in Doctor Napier's "Principles and Practice" which constitutes valuable information in the field of tropical medicine. Certain chapters or portions of chapters must be evaluated in the light of better or more modern presentation of the subject in recent textbooks, monographs and original papers in medical and allied journals. The most noticeable discrepancies in subject matter are (1) too brief consideration of the typhus group of fevers, (2) inadequate presentation in mycology and (3) no section devoted to medical entomology. Another criticism is that the literature of the last five years has been rather consistently neglected, even in the latter part of the volume which was printed anew in the United States. Moreover, there is repeated evidence of the author's inability to accept the newer concepts and perspectives in medicine. The diseases with which the author has had experience in India have been presented quite satisfactorily, while those with which he has not had prolonged, intimate contact have suffered in presentation. The volume lacks balance and coherence. This may be due to Doctor Napier's difficulty in deciding exactly what should be included in a manual of tropical medicine and how much

space should be given to each subject. Finally, it lacks the most essential background for a useful textbook or reference book, namely critical editing. These shortcomings make it impossible for the reviewer to acclaim the volume as a notable contribution to the theory and practice of disease in warm countries.

ERNEST CARROLL FAUST

*Insect Microbiology*, by Edward A. Steinhaus, Assistant Professor of Bacteriology and Assistant Insect Pathologist to the Agricultural Experiment Station at the University of California, Berkeley, California. X and 763 pages, with 250 illustrations. Comstock Publishing Company, Ithaca, N. Y.

As the author states, in a sub-title, this work is "An account of the microbes associated with insects and ticks with special reference to the biological relationships involved." It is written by an authority who is well known because of his contributions to subjects considered in this book and in writing it he has placed upon record an enormous amount of data which should prove of the greatest value to all interested in the subject.

The main body of the book is divided into the following chapters: Specific Bacteria Associated with Insects; Intracellular Bacteriumlike and Rickettsialike Symbiotes; Rickettsiae; Yeasts and Insects; Fungi and Insects; Viruses and Insects; Protozoa and Insects; Protozoa in Termites; Immunity in Insects, and Methods and Procedures. There is a bibliography and an index of authors and of subjects.

In the chapters devoted to the relationship of specific organisms and insects, each organism is considered separately, and the data regarding its association with insects is given in detail, thus affording one access to most of the important work that has been accomplished in regard to relationship of organisms to their insect hosts, methods of transmission of these organisms to animals and man, and much other data of importance.

Within the limits of a review it is obviously impossible to consider *in extenso* the mass of valuable data presented in this book but it may be stated, without exaggeration, that nowhere else in English will one find so complete and accurate a record of most of the subjects considered. However, it may be stated that in view of their importance, the discussions upon amebiasis and malaria appear inadequate. In the consideration of the relation of insects to amebiasis very little data are given and the work of Thomson and Thomson (1916), Wenyon and O'Connor (1917), Roubaud (1918), Root (1921), Frye and Meleney (1932), and Alexander and Dansker (1935) upon the relationship of flies to amebiasis is not mentioned nor is that of Pipkin (1942). In fact, the chapter upon "Protozoa and Insects" is a great disappointment when compared with others in the book, for even so important a subject as the relationship of mosquitoes and malaria is much too briefly considered and a vast amount of research work which has added greatly to our knowledge is not mentioned. The entire consideration of what is probably the most important of all of the insect transmitted diseases, i.e. malaria, covers only seven pages. It is noted that no mention is made of the exo-erythrocytic cycle of development of the malaria plasmodia and credit is not given Craig for the discovery and first description of *Plasmodium ovale*, in 1909, although he did not name it. The absence of any illustrations of the various species of plasmodia detracts from the value of this discussion of the subject. While the author states that he is considering the subject briefly, he has done so much too briefly to render his discussion very valuable and it is certainly not up-to-date in many respects.

It is noted that in discussing the Leishmania the author lists *Leishmania donovani* var *infantum*, as a good variety, but this is not accepted by most authorities and *infantum* is considered as a synonym of *donovani*. In the consideration of dengue fever much valuable data regarding the transmission of the virus by mosquitoes is omitted and the mechanical transmission of the virus by these insects, first proven by Ashburn and Craig (1909) is not mentioned nor are these investigators credited with the discovery that dengue is caused by a filterable virus and the contribution of much data relating to this virus.

Except for the subjects mentioned above the book is very complete and can be cordially recommended to public health officers, entomologists, research workers and all who are

interested in the relationship of insects and pathogenic and non-pathogenic organisms. It should prove of special value to the public health worker and physicians interested in preventive medicine and as a work of reference for students and practitioners of medicine. The addition of illustrations to the discussion of amebiasis and malaria will greatly increase the value of the book as will a more extended discussion of these subjects. The book is beautifully printed and bound and the illustrations are excellent. The Bibliography, as a whole, is good, but many important omissions are noted.

CHARLES F. CRAIG





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# HUMAN TRYPANOSOMIASIS AND TSETSE-FLIES IN LIBERIA

By EVERETT P. VEATCH, M. D., JOSEPH C. BEQUAERT AND DAVID WEINMAN, M. D.

## PREFACE

The various aspects of the Sleeping Sickness problem in Liberia, reported upon in the present joint paper, were investigated at the behest and with the financial support of the Firestone Plantations Company, under the auspices of the American Foundation for Tropical Medicine, Inc., and of the Department of Comparative Pathology and Tropical Medicine, Harvard Medical School and School of Public Health. Dr. D. Weinman and Dr. J. Bequaert, at the time on the staff of the Department, were granted special leave of absence for the purpose by the Corporation of Harvard University. The third member of the party, Dr. E. P. Veatch, carried on his work under contract with the American Foundation. In the authors' well-considered opinion, the results of the work, presented herewith, clearly show at least the need and timeliness of the investigations.

The field work in Liberia covered about a year, from the end of 1943 to the latter part of 1944. Dr. E. P. Veatch reached the country December 1, 1943, and departed at the end of November, 1944. Dr. J. Bequaert arrived with Dr. Veatch and left Liberia August 16, 1944. Dr. D. Weinman resided in Liberia from February 26 to August 12, 1944.

*Acknowledgments.*—We desire to express our gratitude in the first place to Mr. Harvey S. Firestone, Jr., and Mr. B. H. Larabee, President and Vice-President respectively of the Firestone Plantations Company, not only for their generosity and initiative in making the investigations and their publication possible, but also for their continued interest in the progress of the work. We are also under particular obligations to the personnel of the Firestone Plantations in Liberia, especially to Mr. R. E. Wilson, General Manager, for their hospitality and great assistance.

To the Officers of the American Foundation of Tropical Medicine, Inc., we are indebted for entrusting us with the project and for effective support throughout the work in the field, as well as during the preparation of the reports. We wish to mention particularly Dr. Jean A. Curran, Dr. Henry E. Meleny, Dr. Thomas T. Mackie, Mr. L. T. Melly, Mr. E. Gamache and Mrs. C. James Atarian. Colonel Chas. F. Craig made it possible to have our work published as a Supplement to the widely circulated American Journal of Tropical Medicine.

During our stay at the Harbel Plantation, we were the guests of the Medical Staff of the Firestone Plantations Company, who provided us with laboratory and hospital facilities. We are particularly indebted for these to Dr. James L. Doenges, Dr. A. G. Hyde, and Dr. K. H. Franz. Dr. Franz coöperated in the experiments with new drugs, so that his name appears as a joint author of Dr. Weinman's section.



The successive Presidents of the Republic of Liberia, his Excellency Edwin Barclay and his Excellency William Tubman, as well as Dr. Leo Sajous, Medical Officer of Health of Liberia, took a great deal of interest in our work. As a result we were granted official permission to travel in the interior of the Republic for our studies. Mr. Lester A. Walton, American Minister at Monrovia, was most helpful in these matters.

At Bolahun, Fathers Leo F. Kroll and Joseph Parsell, of the Holy Cross Mission (Episcopal), did much to facilitate Dr. Veatch's work in their district. Several other missionaries in the Western Province were also of assistance, particularly Mr. R. H. Embree, at Kakata; the Reverend Wesley Sadler and Miss Esther Bacon, of the American Lutheran Mission at Zorzor; and the Reverend Heilman of the American Lutheran Mission at Sanoyea.

Chiefly owing to the interest taken in the project by Colonel Richard P. Strong, the U. S. War Department extended to us many courtesies, without which our investigations would have been greatly hampered by the then prevailing war conditions. Much valuable help was received from the Commanding Officers of the American Army contingent on duty at the time in Liberia. In addition, the African Army Command made it possible for Dr. Weinman and Dr. Bequaert to visit the Gold Coast and the Belgian Congo for comparative studies of the disease and its vectors.

We acknowledge with thanks valuable help received from the medical authorities of some British and Belgian colonies. In Sierra Leone, Dr. Lightbody, Dr. L. Peaston, Dr. R. D. Harding, the present Sleeping Sickness Officer, should particularly be mentioned. Dr. Veatch and Dr. Bequaert were most courteously received by Dr. and Mrs. Harding during a brief visit to the Kailahun District of Sierra Leone in February, 1944. Dr. Veatch in April, 1944, went there again, meeting both Dr. Harding and Dr. Meunier, of the Medical Service of French Guinea. These joint discussions of the Sleeping Sickness problem in the border areas of Liberia, Sierra Leone and French Guinea were most instructive. In the Gold Coast, Dr. Weinman and Dr. Bequaert discussed the local Sleeping Sickness situation with Brigadier G. M. Findlay and his associates, who were unsparing with their time and facilities. In the Belgian Congo, Dr. Weinman and Dr. Bequaert were received most cordially by Dr. L. Van Hoof, Head of the Medical Service, Dr. M. Wanson, Dr. C. Henrard and Mademoiselle E. Peel, who were all engaged in important research on trypanosomes and tsetse-flies. Dr. Van Hoof was instrumental in having arrangements made by the Belgian authorities for a most illuminating visit to a newly discovered and heavily infected Sleeping Sickness area in the region of Luluabourg. On this occasion, Dr. Weinman and Dr. Bequaert were the guests of the Reverend Washburn and his co-workers at the American Presbyterian Mission, at Bulape, and of Mr. and Mrs. Albert Jacques at Belo, on the Lubudi River.

At the Harvard Medical School, the Liberian investigations were the special concern of Dean C. S. Burwell, Dr. René Dubos, for some time Head of the Department of Comparative Pathology and Tropical Medicine, Dr. D. Augustine, at present Acting Head of the same Department, and Dr. George C. Shat-

tuck, Clinical Professor of Tropical Medicine. Dr. Shattuck has acted as scientific advisor throughout our investigations. We owe a great deal to his unflinching interest, his advice, and his helpful criticism while the work was in progress, as well as during the preparation of the present report.



## PART I

# HUMAN TRYPANOSOMIASIS IN LIBERIA, 1941-1944

By EVERETT P. VEATCH, M.D.

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### HISTORY OF TRYPANOSOMIASIS IN LIBERIA

The first authentic record of trypanosomiasis in Liberia is by the Harvard Expedition in 1926, when Dr. Max Theiler examined 10 children in the towns of Bakratown and Paiata and found trypanosomes in material aspirated from the cervical lymph glands in 5 cases.

Dr. G. W. Harley, in an article "Ganta Dispensary Patients", published in 1933 in the American Journal of Tropical Medicine, records 12 cases of trypanosomiasis from the Central Province, near the French border of Liberia. From his description some of the cases must have been moderately advanced.

My interest in trypanosomiasis began in 1934, when a schoolboy died in the town of Sobobo, on the Kru coast near Sasstown, with typical symptoms of trypanosomiasis. Laboratory examinations were made on blood and spinal fluid, but with negative results. Later, in 1936, when employed by the Firestone Plantations Company at the Cavalla Plantation, my chief surgical assistant, Thomas Brown, a Kru boy, developed clinical symptoms of trypanosomiasis. He had been in school at Freetown, Sierra Leone, for several years and had returned to Liberia in 1932 in good health. He developed enlarged cervical lymph glands, lassitude and lethargy. I failed to find the trypanosomes in the gland material or in the blood. His spinal fluid cell count was 250 cells per c.mm. He was ill four months with typical symptoms of trypanosomiasis and later developed Cheyne-Stokes respiration and died.

The loss of my most valuable assistant stimulated my study of trypanosomiasis. I reviewed all the material available on the subject and determined to find the organism in future cases. The following is a summary of my findings in three cases in the Cavalla River area.

## CASE HISTORIES

Broubo, tribe Grebo, male, age 16. Admitted to the Firestone Cavalla Hospital on December 17, 1937, with a temperature of 100.3°, pulse 120, in a stage of advanced somnolence. Hemoglobin 55 per cent; erythrocyte count 2,670,000. Examination of urine showed it to be normal. A spinal puncture was done and trypanosomes were found in the spinal fluid. Four days later the patient died.

Wata, tribe Grebo, male, age 10. Admitted to the Firestone Cavalla Hospital February 10, 1938. Patient lived near the Cavalla River. He slept most of the time and was carried in by the Chief of Gedetarbo, with a temperature of 100° and pulse of 110. A puncture of the cervical lymph glands was done and trypanosomes were found in the material obtained. The patient died the following day.

Dueh, tribe Kru, male, age 12. Admitted to Firestone Cavalla Hospital April 26, 1938, for a deep sleep from which he could be roused only with great difficulty. Temperature 102°, pulse 100. He had extremely large cervical glands. Trypanosomes were found in the thick blood smear. The patient died four days following his admission to the hospital.

In November, 1937, a visit was made to the French Ivory Coast, where I talked with the French Medical Officer at Tabou. He stated that cases of trypanosomiasis were frequently seen there and that he had 5 patients under treatment at that time. This increased my interest in the disease, as it seemed unlikely that trypanosomiasis would occur on one side of the Cavalla River and not on the other.

The three cases described above were reported to the Firestone Plantations Company and to Dr. G. Fuszek, at that time Director of Public Health and Sanitation for the Republic of Liberia. The blood slides containing the trypanosomes were demonstrated to Dr. F. Mouri, Medical Officer of Health of Maryland County. Dr. Fuszek informed me that a doctor previously employed at the Cavalla Plantation had found one case of trypanosomiasis there. He also stated at that time that trypanosomiasis did not occur in Liberia, but that a few cases had been imported from British and French colonies, the Spanish island of Fernando Po and the Portuguese island of St. Thomas. He stated that native laborers who went to work at the cocoa plantations of St. Thomas and Fernando Po had probably introduced trypanosomiasis into the coastal region of Liberia. Large numbers of these laborers were sent from Liberia to these islands following the World War, and when they returned they were in very poor health. The agent for the Spanish and Portuguese Steamship Company stated that many of these laborers lay on the deck of the ship in a semiconscious state. Some of the laborers with whom I talked after their return from the island of Fernando Po stated that many of their companions had died of this sleeping sickness during their stay on the island or on their way home.

The first known writing giving information about medical practice in Liberia is "History of Africa Missions of the Protestant Church" by Mrs. E. F. Hening,

published in 1850. The material for the book was obtained from the letters of Dr. Savage who spent seven years (from 1843 to 1850) in Cape Palmas, Liberia.

He was a graduate of Johns Hopkins University, and gave a clear clinical description of many of the diseases he observed. A very careful study was made of his cases to find out whether any contained clinical symptoms of trypanosomiasis, but none were found. However, most of his cases were illnesses of the white staff who had been only a short time in Liberia. He describes yellow fever, malaria and dysentery. Of the 16 cases described on one tour of service, 7 died and 3 were sent home invalided, but 6 were able to stay on and continue to live in that area.

One of the best evidences that trypanosomiasis is endemic in Liberia is the fact that there is a word for trypanosomiasis in most of the native languages. However, native people do not associate the disease with the presence of the tsetse-fly. The native Medicine Man, or Zo, can give a very accurate clinical picture of trypanosomiasis.

The native names for trypanosomiasis are as follows:

In Gbandi—Gechaibehe

In Kissi—Nar-maloh

In Buzzi—Nyewenezibeh

In Kpelle—Yekolofia

In Bassa—Nyeonmoweh

In Kru—Nyono-kweh

The native names given to the tsetse-flies are as follows:

In Gbandi—Dehowologi

In Kissi—Yollo

In Buzzi—Defowologi

In Kpelle—Meileh

In Bassa—Wloo

In Kru—Giekawloo

I inquired of the native people in 1932 whether the disease Nyono-kweh (Kru name for sleeping sickness) had occurred in the Kru tribe in the past. George Watte, an old Kru man, stated that the disease was present on the Kru coast in his grandfather's time, but that there were few deaths in any one year. The native custom was to drive people from the town if they showed signs of stupor and to force them to live in the rice kitchens at the farms, where they usually died of neglect.

When cases were later found at the Firestone Cavalla Plantation, the Grebo chiefs of Gedetarbo and Webbo were asked if sleeping sickness occurred in their country. They replied that the condition was the same as in Kru country and that the same word was used for sleeping sickness by the Grebo tribe as by the Kru.

In "The African Republic of Liberia and the Belgian Congo", by Richard P. Strong, an account is given of a Vai tribesman, Doala Bukere, who invented the Vai alphabet. It is believed that he died of sleeping sickness. Sir Harry Johnston also gives a colored photograph of a Mandingo of healthy appearance who, he states, died of sleeping sickness afterwards in 1905.

The Vai and some other tribes refer to the disease as "ball sickness". This is interesting because the Vai tribe originated an operation to remove the enlarged glands in the neck. The art of the operation was learned later by the

Buzzi Medicine Men and at present it is performed widely in this tribe. The operation is so common that, in making a sleeping sickness survey of the Buzzi tribe, the presence of scars on the neck of a number of people was strongly suggestive of trypanosomiasis. I have treated many patients who had from one to six of the cervical glands removed. The native theory is that removal of the glands will cure the disease. The operation is a simple one. The surgeon holds the enlarged gland between his thumb and finger and makes an incision over the gland one inch or more in length. The exposed gland is then caught by an instrument similar to a fish hook and dissected out with a blunt instrument. The control of hemorrhage is by an adaptation of the circumcision technique. The bleeding skin edges are pressed tightly together, using two pieces of bamboo, the firm pressure stopping the hemorrhage. After the bleeding has been controlled, charcoal or white clay is applied to the wound. No anesthetic of any kind is used in these operations. Infection occurs in a few cases, but most wounds heal within a short time. The native people state that this operation has been performed among them for many years. Since it is painful and expensive, even when done by a native Medicine Man, patients do not object to coming and taking a course of tryparsamide treatment instead of undergoing the operation.

Although Winterbottom observed cases of trypanosomiasis in 1803 near Sierra Leone, the disease must have remained there at a low level of infection rate until the close of the World War. About that time more interest was given by the British and French governments to the native affairs in the interior of their colonies, and more supervision exercised. Developments were begun and trading stations opened up in many parts of the interior. Gradually roads and railroads were built, which made travel commonplace, so that tribesmen went long distances. In the old days tribal wars and boundary disputes kept the natives within their own tribe limits or villages. These barriers blocked off the country into small areas and localized epidemics. The establishment of large cities along the coast and the development of the natural rubber industry in Liberia broke down these old barriers, so that epidemics were no longer local. While there is little or no road development in this part of the Republic of Liberia, the French and British roads are within a few miles of the Liberian border and the commerce of these two countries affects the interior tribes of Liberia. The portage system of Liberia and the recruiting of labor for the rubber plantation has taken many natives from their local familiar surroundings into a new environment. Thus the development of roads and industry and the stopping of native wars have produced new social and economic influences. Trypanosomiasis is now no longer a disease affecting a few tribesmen, but has been increasing at an alarming rate. Since without the help of native laborers the wealth of West Africa cannot be developed, it is necessary for the governments concerned to control the disease.

The French colonial officials have been aware of trypanosomiasis in West Africa for many years. They began control measures in French Guinea in 1930 and have continued them consistently ever since. This colony forms the north-

ern boundary of the Republic of Liberia. The native Kissi and Buzzi tribes are found in both countries and health conditions are about the same in the general area occupied by these tribes on both sides of the boundary. A central laboratory of the trypanosomiasis service was established in Gueckedu, French Guinea, and the whole population has been examined several times. Gueckedu is 3 miles from the Liberian border and probably the geographical center of the Kissi tribe. A European doctor, with a well-trained staff of native assistants, has been at work there continuously since the beginning of 1930. For a number of years every native entering French Guinea from Sierra Leone or Liberia was examined. Probably more than one-third of the Kissi tribe live in French Guinea, about one-third in Liberia, and less than that number in Sierra Leone. They occupy an area of land often described as being the shape of a vermiform appendix, approximately 3 miles in width and more than 22 miles long. In this area, the French physician in charge of trypanosomiasis work noticed that it was impossible to reduce the incidence of trypanosomiasis, while in other sections of French Guinea, as, for instance, around Gueckedu, the rate of infection had been steadily reduced. It was his opinion that cases were coming into this area from the surrounding British colony of Sierra Leone. The attention of the British Government having been called to this fact in 1936, an investigation was started by Dr. W. Williams, Medical Officer of the Kailahun District of Sierra Leone. Cases of trypanosomiasis were found and the work of control was first organized in Sierra Leone under Dr. Williams.

In 1940, Dr. R. D. Harding who had been for many years in Nigeria in trypanosomiasis service, took over. He has published a great deal on trypanosomiasis both in Nigeria and in Sierra Leone. In his paper reviewing trypanosomiasis from January to April, 1944, he states that the incidence in the Kissi Kama and the Kissi Teng sections was 20.3 per cent in 1940. This section of Sierra Leone forms the boundary of Liberia. The first British training center for teaching assistants was located at Quendu, Sierra Leone, 3 miles from the Liberian border near the town of Sodu, Liberia. Quendu was about the center of the Sierra Leone epidemic. Sodu had the highest rate of trypanosomiasis infection found by me in any town in Liberia in 1941. Sierra Leone has maintained a trypanosomiasis unit under Dr. Harding since that time, and while the rate of infection is now less than 1 per cent, except in one small area where intensive work is now in progress, resurveys are done regularly to find any new outbreak.

In 1940, Dr. J. S. Seldon, Director of Public Health and Sanitation for the Western Province, Liberia, made a survey at the request of Dr. R. G. Fuszek, then Director of Public Health and Sanitation of Liberia. He was employed by the Holy Cross Mission of the Protestant Episcopal Church. Following a survey in Kissi country, Dr. Seldon reported that there were many cases of trypanosomiasis in all sections of this area. He diagnosed 42 cases at his clinic of the Holy Cross Mission Hospital in Bolahun and treated them.

Dr. Leo Sajous, Medical Officer of Health for the Liberian Government in charge of trypanosomiasis, arrived at the Government Station of Kolahun, in the Western Province, early in 1941.



The author arrived in the Western Province, Republic of Liberia, in March, 1941, sent by the Firestone Plantations Company to assist the Health Department of the Republic of Liberia with the problem of trypanosomiasis. The needed supplies, drugs and equipment were also furnished at the same time by the Company.

The first stage of my program was to train young natives to use a microscope and assist with the laboratory and clinical work. Later on other natives were trained in certain phases of treatment. A period of three months was spent at the first station in training, and then another three-month period at the second station. During these first months frequent trips were also made to Sierra Leone to observe the diagnosis and treatment units. Dr. R. D. Harding, at that time in control of the work in Sierra Leone, was very helpful as a teacher, giving all possible assistance.

The local government of the Interior is under a District Commissioner, appointed by the President of the Republic of Liberia and assisted by the Paramount Chiefs. Each tribe has a Paramount Chief, assisted by five Clan Chiefs. In the Kissi tribe the Paramount Chief has approximately 23,000 people in his chiefdom. The various Clan Chiefs have from 4,000 to 6,000 people each in their clans and are assisted by the town chiefs. There are from 40 to 60 towns in each clan and each town has its own chief.

In a trypanosomiasis campaign the best unit of the population to deal with is the tribal clan, which represents approximately 4,000 to 6,000 people. This unit of government is headed by the Clan Chief and the people are used to his authority. The entire tribe is too large a unit and a town is too small to be convenient in handling large numbers of cases of the disease. The best place to start diagnosis work is the town where the Clan Chief resides. The next step is to ask the Clan Chief to furnish a list of the towns in his clan, with the approximate number of houses in each town and the name of the town chiefs.

*Method of Examination and Treatment in 1941-1943.*—At the end of the training period about 100 laboratory examinations could be made each day, with the help of the assistants. The Clan Chief was asked to call all the people of a town or several towns, depending on their size. Two hundred to 400 persons could be seen daily. When a group of people was gathered, they were placed to form a line beginning some 20 feet from the examiner's station. At a signal to the assistant at the head of the line, a native is sent toward the examiner. As the person approaches the examiner his gait is carefully noticed. A change in coördination can be observed in early cases of trypanosomiasis. The patient is then examined carefully for any enlarged glands. He is placed with his back to the examiner, the cervical glands being first palpated, then the epitrochlear and inguinal glands. Special care is taken to observe change in size in the posterior cervical group, because this is the group where the first swelling usually occurs. The patient's temperature is noted during palpation and a reading taken if there seems to be any question about it. High fever can easily be found in the regular course of examination. All persons who give no signs or symptoms of the

disease clinically are marked negative and allowed to return to their homes. Those who have fever, glandular enlargements or nervous symptoms suggestive of sleeping sickness, are kept for laboratory examinations and divided into two groups by marks on their chests. Those with glands large enough to be punctured are marked +. All others are marked P for positive. The serial number in the examination is also marked on the patient's chest; for instance, the first patient would be +1 and the second P2, and so on down the series. The patients now go to the clerk's table, where the following records are made: name, age, sex, father's name, Paramount Chief's name and Clan Chief's name.

From there the patients are sent to the table for taking blood slides and the same number is marked on the slide as on the patient's chest. A thick blood slide is made with *three* thick drops on one slide. The patient then goes to the waiting shed, where he remains until called. Blood slides are taken of all cases. After drying, the thick film preparations are dehemoglobinized and stained with Giemsa stain. When dry again, the slides are ready for examination.

While the staining and drying of the slides is in progress, the microscopic examination of the enlarged glands is started. In the waiting shed the cases marked + are separated from the others and gland punctures are done on all of these. As soon as a microscopist finds a trypanosome in the fresh gland fluid, the doctor is called to confirm the diagnosis. If the patient is found to be positive, a history is taken and a physical examination done, after which he goes to the treatment department where he receives his first treatment and is told to return in 5 days. His blood slide is then removed from the other slides, because a diagnosis has already been made. When all the gland punctures have been completed, the examination of the thick film preparations is begun.

Usually the trypanosomiasis station was constructed in the Clan Chief's town. A clearing approximately 400 yards from the town was made large enough to accommodate the diagnosis and treatment sheds, constructed of native poles and roofed with palm fronds. The sheds were 15 by 25 feet and usually four of them were constructed. Stick fences were built to make it easier to keep patients in line and to lead them to the places where they were needed. The cost of construction of these diagnosis and treatment units was about \$12.00 to \$15.00 for the four sheds. Ordinarily, this center was used for three weeks or more for the diagnosis unit, and for ten to twelve weeks for the treatment unit.

The following factors are significant in getting native people to return for treatment. Stations must be so located that a native man will not have to walk more than two hours to get his treatment, which should be available five days a week at these stations. It is very difficult for a native man to remember the five-day period, but usually he will return the same day of each week. He is very reluctant to leave his clan and enter another clan for treatment.

During the training period, ten young natives were trained to use microscopes. They were taught how to search for the active trypanosomes in the fresh material obtained by gland puncture, and for the stained trypanosomes in a thick blood smear. They assisted in such work as numbering the slides and staining. In

addition, five young natives were trained to do a very simplified form of treatment. The most difficult part was to teach them to do intravenous injections, but this was accomplished satisfactorily after a period of training.

During this first term of service in the Western Province of the Republic of Liberia, many difficulties were encountered in connection with the work. The first problem confronting us was the total lack of census of the country, no one knowing what the population really is. The only rough scale to judge by is the number of huts on which the Government collects taxes. This is not a very reliable index, as there are "ghost towns" consisting of a few huts in a dense wooded area where a few people live, the location of such towns being closely guarded with secrecy in order to avoid paying taxes.

The training of assistants is a difficult problem in the interior of Liberia, where there are no schools which teach above the fifth elementary grade. Young men from the coast are not content to work in the interior, as it is like a foreign country to them. The interior is ruled by one man, the District Commissioner, and unfortunately he has had little or no public health education. The police help furnished by the Government are not well trained nor very dependable. There are also many trying problems in dealing with native labor.

During the first term of service (1941-1943), which lasted 26 months, a total of 90,980 persons were examined clinically for trypanosomiasis. There were 31,397 laboratory examinations made during the campaign and 13,481 cases of trypanosomiasis diagnosed, 6,803 of them males and 6,678 females. In addition, 91,935 single treatments were given: 4,452 patients or 33 per cent had less than six treatments each, and 9,029 cases or 67 per cent received more than six treatments. There are probably more than 100,000 people in the Vonjama-Kolahun area where this work was done. It should be noted, however, that the total number of people examined and the large number of cases found in 1941-1943 does not give the true rate of incidence of the disease, because sick people come in from outside the area, some traveling 100 to 150 miles in order to get treatment. No other physician was available in Liberia for approximately 180 miles. The patients came from all tribes, but the Kissi were first in importance and the Gbandi second.

The epidemiology of trypanosomiasis in this part of West Africa is associated with the habits and movements of the Kissi tribe of native people. The British in Sierra Leone and the French in French Guinea have found large numbers of their cases among the Kissi people. The problem of trypanosomiasis in these countries centers in the Kissi tribe. The Kissi people know no international boundaries and move freely from one country to another to visit relatives and friends. The close association of the Kissi tribe with the Gbandi tribe probably accounts for the high incidence among the latter tribe, and the same is true of the small Mendi tribe.

The next phase of the trypanosomiasis work in northwestern Liberia was the survey done under the auspices of the American Foundation for Tropical Medicine, Inc., by Dr. Joseph C. Bequaert and myself from December, 1943, to March, 1944. The results of this survey are covered in the remainder of the present report.

Dr. G. C. Campbell reported at the Trypanosomiasis Conference at Lagos, Nigeria, in July, 1943, that the infection rate for trypanosomiasis on the Harbel Firestone Plantation in Liberia was from 0.25 to 1.0 per cent. From June, 1943, to November, 1943, 266 cases of the disease were diagnosed at the Harbel Plantation, according to Dr. James Doenges, who was at that time in charge of the medical work of the Plantation.

#### GENERAL SUMMARY OF THE WORK

During the time I was employed by the American Foundation for Tropical Medicine, Inc. 32,617 persons were examined clinically. This figure includes patients seen on all the cursory survey trips and during the regular diagnosis work in Kissi country, about one-third the number of patients I saw during the previous period of work from 1941 until May, 1943. This population includes all the tribes of the Western Province: Kpesi, Belle, Mendi, Buzzi, Mandingo, Gbandi and Kissi. The facts about the different tribes will be considered later. The group was not selected in any way, but represents a random cross-section of the population for this part of Liberia.

The group considered includes 14,462 males and 18,155 females, or 3,693 more females than males, the ratio of males over females being 0.7965 on the per capita basis. This division of the population is accounted for in several ways. There are probably about as many males as females in Liberia as a whole; but the male population is greater on the coast and the female population greater in the interior. Many men go to the coast and engage in work on the plantations or in industry, returning home about once a year. There are many reasons why the women stay in the interior and do not go into the industrial areas. The best land for rice farming is in the interior and women do a large part of the farming. Women are an economic asset on the land and a liability to the family in the industrial areas. Because of the division of labor among native people more women than men are required to produce rice. This condition of more females in the interior is likely to continue because it is based on certain native customs. Recruiting of laborers for industry continues all the time.

Three laboratory procedures are commonly used in the diagnosis of trypanosomiasis. Gland puncture comes first in importance. The examination of a preparation of blood known as the thick smear comes next. The third procedure is the examination of spinal fluid. I shall deal only with the first two methods in this part of the paper.

During 1943-1944, 7,985 laboratory examinations were made, of which 2,117 were from material obtained by puncture of cervical glands and 5,866 were on thick blood film preparations.

Of the 2,117 examinations from material obtained by gland puncture 1,152 were of male and 965 of female patients. Trypanosomes were found in 501 of these cases and 1,616 smears were negative. In 69 per cent of all cases a diagnosis was made by the gland puncture method.

Of 5,966 examinations of thick blood smears, 3,148 were of males and 2,718 of females. Trypanosomes were seen in 217 cases, there being 2718 negative blood slides. Thirty per cent of all cases were diagnosed in this manner.

In a population of 32,617 patients, seen during my term of service, 718 cases of trypanosomiasis were found.<sup>1</sup> The over-all rate for all patients examined and positive cases found is 2.20 per cent, which is not a high rate. It applies to a section where a survey was made after an intensive treatment program had been carried out, as well as to some areas where trypanosomiasis was not known to be prevalent. This over-all rate does show that trypanosomiasis is of public health importance in the Western Province of Liberia.

#### SURVEY ON THE INCIDENCE OF THE DISEASE IN THE WESTERN PROVINCE, LIBERIA

The survey began on December 20, 1943, at Dobli Island and continued until March 22, 1944. Its purpose was to determine as nearly as possible the infection rate of trypanosomiasis in the Western Province. Previously work had been done only in the extreme Northwestern corner of the Province. Cases had reported themselves for treatment from all over this large area, but little or nothing was known of the prevalence of the disease. The survey was necessary in order to obtain the facts needed for an estimate of the public health significance of trypanosomiasis in this area, and in order to correlate the finding of the disease in a population with the findings of the entomologist. Dr. Bequaert was making a study of the vector of the disease at the same time.

The survey was done along the great trails which connect the interior with the coastal roads. If a disease localized in the interior begins to spread, it would first be noticed in the towns along these trails, where a large number of people travel each year. The area covered is important because of its close contact with the British and French Colonies.

Due to the fact that the British Colony of Sierra Leone has developed roads and a railroad which extend nearly to the Liberian border, there is a great deal of travel from Liberia to Sierra Leone. The French in the Colony of French Guinea also have developed a road system along the Liberian border. Trading posts occur along this border and the natives travel across it for the purpose of trade. Another fact that makes for travel in this area is that one-third of the Kissi tribe live in Sierra Leone, one-third in French Guinea and the other third in Liberia. The natives visit relatives in all three countries. The French Government has actually engaged in trypanosomiasis work for 13 years. The Sierra Leone Government has had special medical officers for this work for 6 years. General health conditions are about the same in all three countries.

The survey covered large areas of territory, with many types of country. Some of it is thickly populated, while other parts have very few inhabitants. It may be said in general that the population in Liberia is greatest in the coastal area and along the French border. Between these two areas there are stretches where very few people live, some of the country being covered by virgin forest. In general the area is rough, with many rocks and hills, and gradually rises in elevation from the Coast to the interior. Where there is a large population the

<sup>1</sup> This total does not include 78 cases which were treated at Bolahun, but which could not be located according to town.

forest has been cut many times for making rice farms, small trees and undergrowth being then the main types of vegetation.

The general plan of the survey was to travel at first due north through the Western Province. This part of the journey began at Lakata in the Central Province and ended at Bolahun in the Western Province. Two short journeys were then outlined to make a survey of the Kissi and Gbandi tribes from this center. A longer journey was planned in the Buzzi section, taking us through Kolahun, Vonjama, Pandamai and Zorzor. At this point Dr. Bequaert returned to the Firestone Plantation at Harbel, while I returned to Bolahun through an area of Buzzi country not yet studied. In all, four weeks were taken to study conditions in the Buzzi country, work being done in 13 Buzzi towns.

The entire survey covered nearly three and one half months and studies were made in 31 towns, located in nearly all parts of the Western Province.

The survey began on the 22nd of December, 1943, at Dobli Island, in the St. Paul River. The people of this town are of the tribe which I shall refer to as the Kpesi. There is much confusion about this name, as the tribe is variously called Kpesi, Kpelle, Kpees, Pessie and Pelle.

TABLE I  
*Survey of the Kpesi Tribe*

DATE	TOWN	NUMBER OF HUTS	POPULATION	POSITIVE CASES	PER CENT
12/22 to 3/43.....	Dobli Island	119	232	3	1.29
12/27 to 8/43.....	Zowolata	111	449	0	—
12/30 to 1/43.....	Kumbaeta	105	239	4	1.67
Totals .....		335	720	7	0.986

The first three towns in our survey were in Kpesi country, namely the towns of Dobli Island, Zowolata and Kumbaeta. The Kpesi tribe, located on the first plateau, begins about 50 miles inland from the sea and extends to the range of hills near the town of Belleyella. It extends in width from the Gola Forest to the St. Paul River. The tribe is a large one, and has a great deal to do with the people of the coast and very little communication with the tribes farther inland. The country is covered with small trees and low bush. The land has been cut over many times for the making of rice farms.

An examination of 720 persons was made in these three towns, seven cases of trypanosomiasis being found. The infection rate of this group is 0.98 per cent (Table I). Since the rate of infection found in this area is less than one per cent, trypanosomiasis cannot be considered of public health importance. However, it must be pointed out that all the cases were residents of this area and were not strangers who had come in from the interior tribes.

The next part of our survey was in the Belle tribe. This is a small tribe, of the same anthropological group as the Kru people, who occupy the sea coastal region in the central part of Liberia. Their language is similar to that of the

Kru people. There are only 15 Belle towns. The Collector of Internal Revenue at Belleyella stated that the Belle tribe paid taxes on 1120 huts. There are probably 4,200 people in this tribe. The country is extremely hilly and has extensive forests. The towns are widely scattered.

During my first term of service from 1941 to 1943, fifty-five Belle people were found positive for trypanosomiasis. Most of these patients walked 70 miles or more to obtain treatment. In the present survey (December, 1943) work was done in three towns, Belleyella, Fasima and Guawarmarma. In all, 653 persons were examined and 18 positive cases found. The rate of infection is 2.75 per cent (Table II).

The lowest rate of infection was at Belleyella, with 1.36 per cent. This town is a government station and an Army Post and does not represent a cross-section of the Belle population because of the families of soldiers who live there. The highest rate of infection was at Guawarmarma, with 4.54 per cent. This town, the home of the Paramount Chief of the Belle tribe, is near the large Laowa

TABLE II  
*Survey of the Belle Tribe*

DATE	TOWN	NUMBER OF HUTS	POPULATION	POSITIVE CASES	PER CENT
1/2-3/44.....	Belleyella	86	220	3	1.36
1/7-8/44.....	Fasima	116	279	8	2.87
1/10/44.....	Guawarmarma	88	154	7	4.54
Totals .....		290	653	18	2.75

River. The people plant rice farms near the river. It was from this general area that the 55 cases came which were treated in 1942. With a general rate of 2.75 per cent and with a probable population of more than 4,000, there are possibly 110 cases of trypanosomiasis in the Belle tribe. Since the Belle people do not form a very large part of the labor supply for the rubber plantations the loss of man power is not of very great importance economically. Nevertheless, trypanosomiasis is of public health significance in this tribe.

The Gbandi tribe is located in the northwestern part of the Western Province, south of the Kissi tribe and north of the Belle tribe. It pays taxes on 5,862 huts, according to the Collector of Internal Revenue at Kolahun. The rate of infection is low in this tribe, as is to be expected for several reasons. Treatment was carried on from March 24, 1941, until May 20, 1943, during which time 3,425 patients from this tribe were diagnosed and treated. This tribe thus had the benefit of two and a third years of education as to the value of treatment. The four towns used as a sample for the present survey (1944), were Loboba, Massambolahun, Bolahun and Nyandamolahun. Among 1,546 persons examined, 22 positive cases were found. The rate of infection was 1.43 (Table III). Part of the positives of this group were relapsed cases.

It should be pointed out that the rates of infection in the Gbandi country vary a great deal. The clans in the Gbandi tribe bordering on the Kissi tribe have the greatest number of cases of trypanosomiasis, while those farther from the Kissi tribe have the lower rates. The close association with the Kissi tribe is one of the factors in the spread of the disease. With an infection rate of 1.43 per cent and with a probable population of more than 23,000 people, there may be 328 cases of trypanosomiasis in the Gbandi tribe. Trypanosomiasis is decidedly a public health problem in this tribe, and, unless control measures are put into effect, the disease will again become epidemic there.

The Kissi tribe has long been associated with the spread of trypanosomiasis. Part of this tribe is located in Liberia, part in Sierra Leone, and part in French Guinea. In all three countries the rates for trypanosomiasis have been higher among them than among any of the adjoining tribes.

When work was started in Liberia in 1941 the rate of infection was very high in the Kissi tribe. One or two diagnoses and treatment centers were located in each of the five clans of the tribe, the entire population of which was examined

TABLE III  
*Survey of the Gbandi Tribe*

DATE	TOWN	NUMBER OF HUTS	POPULATION	POSITIVE CASES	PER CENT
1/12/44.....	Loboba	78	342	6	1.75
1/23-24/44.....	Massambolahun	238	366	5	1.36
1/21-24/44.....	Bolahun	89	244	10	4.09
1/24/44.....	Nyandamolahun	117	594	1	0.01
Totals .....		522	1,546	22	1.43

between June, 1941, and May, 1942. The rates for this section taken together were at that time 20 per cent of the population examined. In the second half of 1942 a second examination was made of all the Kissi tribe, the rate of infection found this time being 5 per cent. In tabulating all the results of the first term of service, it was found that 26 per cent of all the Kissi population had been diagnosed as having trypanosomiasis. All work was closed in May, 1943, and nothing further was done in this section until January, 1944.

In the present survey (1944), 1,479 persons were examined in six of the larger towns of the Kissi tribe: Kondobengo, Foyakamara, Sodu, Sardu Pascia, Bolelu, and Konosu. There were 37 cases of trypanosomiasis found, many of them relapsed cases. The infection rate in this group is 2.5 per cent (Table IV). From a check of 2,764 cases examined, who had been previously treated in 1941-43, it was found that approximately 4 per cent of the cases had relapsed. The problem in the Kissi section was as much a problem of dealing with the relapsed cases as with new cases.

The problem of epidemiology of trypanosomiasis in this part of Africa has long been associated with the habits and movements of the natives of the Kissi



tribe. They know no international boundaries and move about freely from one country to another to visit relatives and friends.

There are four factors which tend to make for an increase in trypanosomiasis in the Kissi section: (1) The Kissi people raise swamp rice and work in water and close to shade. Their farm kitchens are near the swamp. Other tribes do not raise swamp rice. (2) Kissi people are migratory in their habits. They make long tours every year visiting relatives and friends. (3) Towns are small and there is not sufficient clearing around them. It is not difficult for the flies to travel from the water side to the towns. (4) The Kissi people keep hogs. Van Hoof and other workers believe the hog may be a reservoir host of *T. gambiense* closely associated with man.

There are by actual count 5,420 huts in Kissi country. The population is probably more than 20,000, so that, with a rate of 2.5 per cent of infection, there are probably 500 cases of trypanosomiasis in the Kissi tribe.

TABLE IV  
*Survey of the Kissi Tribe*

DATE	TOWN	NUMBER OF HUTS	POPULATION	POSITIVE CASES	PER CENT
1/28/44.....	Kondobengo	60	272	10	3.67
1/29/44.....	Foyakamara	96	335	8	2.38
1/30/44.....	Sodu	63	178	2	1.12
2/1/44.....	Sardu Pascia	90	329	7	2.43
2/2/44.....	Bolelu	42	165	5	3.03
2/8/44.....	Konosu	82	200	5	2.50
Totals .....		433	1,479	37	2.50

It should be pointed out that the reduction of the rate of infection from 26 per cent in 1941 to 2.5 per cent in 1944 is a step of considerable progress.

The tribe of the greatest importance to us are the Buzzi, referred to as the Loma tribe in many publications. The French call it the Toma tribe in their writings. A large portion of this tribe live in French Guinea, where the center of the tribe is the town of Macenta, which is 8 hours trek from the Liberian town of Vonjama. Vonjama is the headquarters for the Liberian District Commissioner who rules the Buzzi, Gbandi and Kissi tribes.

The area of the interior of Liberia occupied by the Buzzi tribe can be roughly bounded by a triangle. The three points of this triangle are the towns of Kolahun, Vonjama and Zorzor. The actual area is a little larger than this triangle.

The Buzzi are very important economically, as a considerable part of the labor force on the rubber plantations comes from this tribe. It also pays a large part of the taxes in the Western Province. The towns in this tribe are large and there is considerable distance between them. There are more large forests in this section than elsewhere. The land is better for agriculture. There is a good deal of travel from Liberia to the French trading posts and to the town of Macenta in French Guinea.

During our survey of 1944, thirteen towns were visited and the entire population examined. Since our tour took us to practically all sections of the Buzzi tribe, our findings should be fairly representative. Among 5,317 persons examined clinically, 107 cases of trypanosomiasis were found. The rate of infection in this group is 2.01, which is not a high rate (Table V). It should be noted that only two towns fall below the infection rate of one per cent. These are Zorzor and Vasala. At Zorzor a large number of cases were treated in 1942. At Vasala very intensive work was done in January, 1943, and many cases treated. The town with the highest rate of infection was Lormai, with 8.09 per cent. Next in order is the town of Vonjama, with an infection rate of 4.43 per cent. Lazelemai had a rate of 3.65 per cent. These three towns are lo-

TABLE V  
*Survey of the Loma (Buzzi) Tribe*

DATE	TOWN	NUMBER OF HUTS	POPULATION	POSITIVE CASES	PER CENT
2/25/44.....	Vasala	143	317	3	0.94
2/26/44.....	Vonjama	350	632	28	4.43
2/28/44.....	Lormai	78	219	8	3.65
2/28/44.....	Lazelemai	73	210	17	8.09
2/29/44.....	Pandamai	256	573	4	0.69
3/5/44.....	Kazar	88	246	2	0.81
3/6/44.....	Daugomai	142	470	8	1.70
3/8/44.....	Zigida	301	493	7	1.40
3/9/44.....	Bokesa	501	536	10	1.86
3/11/44.....	Zorzor	462	855	5	0.58
3/12/44.....	Zorzor School		152	2	1.31
3/15/44.....	Wauamai	244	399	10	2.94
3/17/44.....	Sobeleva	124	215	3	1.39
Totals .....		2,763	5,317	107	2.01

cated in the same area. There are many other towns in the Buzzi tribe where the rates are low. The only way I can account for this high incidence in the Vonjama area is that Kissi laborers were used to build a government road to Vonjama in 1939 and 1940. At this time trypanosomiasis was very common among the Kissi people. They lived and worked in the Vonjama area for a considerable time.

It may be pointed out that trypanosomiasis is patchy in distribution in the Buzzi tribe. Since the towns are large and widely separated, trypanosomiasis is a town disease in this area.

According to a statement of the Collector of Internal Revenue at Kolahun, the Buzzi people pay taxes on 9,760 huts. This probably means that the population of the tribe is 42,050, and that with an infection rate of 2.01 per cent, there are approximately 841 cases of trypanosomiasis.

In conclusion the survey shows that trypanosomiasis is present over a wide area in the Western Province. The disease is patchy in distribution. The highest rate found was at Lazelemai, where it was 8.09 per cent.

One town was found without a case of trypanosomiasis.

Trypanosomiasis is a disease of public health importance in the Western Province.

#### REVIEW OF TRYPANOSOMIASIS CASES, WESTERN PROVINCE, LIBERIA

A careful history was taken and a complete physical examination made of 796 patients, all diagnosed between December 20, 1943, and November 1, 1944. All patients seen on the various survey trips, or in the intensive campaign in Kissi tribe, or at the treatment center, are included in this group. They came from many tribes and a very wide area of country. Among the 32,617 persons examined on this tour of service, 796 positive cases were found, 419 being males and 377 females. Whereas in the grand total of 32,617, there were 3,693 more females than males, among the positive cases of trypanosomiasis the males outnumbered the females by 42. Thus the ratio of males in the entire group examined is 0.7965 on the per capita basis, while in the case of patients with trypanosomiasis the ratio of males is 1.11 per capita of population. During my first term of service (1941 to 1943) there were also more males who had trypanosomiasis than females, since of 13,481 cases diagnosed at that time 6,843 were males and 6,678 females, giving a ratio of males over females of 1.02 on the per capita basis. It should be noted that during both terms of service there was a great preponderance of females over males in the total number of persons examined. The conclusion seems warranted that in northwestern Liberia trypanosomiasis is a disease slightly more prevalent among males than among females.

There are several reasons why trypanosomiasis is more common among males than among females. The men are exposed to the bite of the tsetse-flies during the time they spend as laborers in carrying loads long distances, as they cross many rivers and swamp land. During the time the men work on roads they are exposed to the bite of flies, while others are bitten when they operate rafts or canoes at the river crossings. Men and women are about equally exposed in the rice farms. Some men spend a great deal of time hunting, and naturally this work takes them along rivers where the flies are abundant. While he is fishing the native is exposed more than anyone else. The rivers are nearly all shaded and this provides a very attractive place for the tsetse-fly. It is likely that men have trypanosomiasis somewhat more frequently than women because the nature of their work exposes them more to the bite of the fly.

The number of relapse cases occurring in this group is large. Of the 796 cases diagnosed there were 113 relapsed cases and 683 new cases. The per cent of relapsed cases is 14.0 to 86.0 per cent new cases.

Between March, 1941, and May, 1943, 7,335 patients had completed eight or more treatments for trypanosomiasis, and 9,026 had received six or more treatments. The number of treated cases in the Kissi and Gbandi tribes was

high in proportion to the population. From an examination of 2,764 patients who had received treatment from March, 1941, to May, 1943, 3.10 per cent of the cases had relapsed. Of the several factors that tend to give a high relapse rate, insufficient treatment is one of the most common. A spinal puncture should be performed and the result recorded before adequate doses of the drug can be prescribed. In doing a mass treatment in the course of a campaign to reduce the infection rate over an area, it is difficult to do a spinal puncture on every case. As a result many cases with great changes in the spinal fluid are so far advanced that the treatment given is not sufficient to bring about a cure. In mass treatment the dose is standardized and often personal attention as to dosage cannot be given to individual cases. Irregularity in taking treatment also causes the drug to be ineffective. Arsenic resistant trypanosomes are often thought to be a cause of a high relapse rate, but I have not found such strains in Liberia. A low standard of living and particularly famine or insufficient food during the period of taking treatment or shortly afterward tend to break the patient's resistance to trypanosomiasis and may also produce a relapse. Other debilitating diseases, such as amoebic dysentery and schistosomiasis, may weaken the patient and cause relapse.

Four methods of diagnosis were used—gland puncture, thick blood film, clinical diagnosis and spinal puncture. Of the 796 positive cases, 541 were diagnosed by gland puncture, 199 by thick blood film, 35 by clinical examination and 21 by spinal puncture.

Many of the clinical cases were confirmed by laboratory findings. Some of the patients were insane, which increases the difficulty of doing laboratory work. Some were too weak to have a spinal puncture done after a long hammock trip.

Sixty-seven per cent of the cases were diagnosed by examination of the material aspirated from the cervical lymph gland, which is the most practical method for examining large numbers of patients. It is the method of choice when using inexperienced microscopists, the mobility of the trypanosome making the diagnosis relatively easy. One-fourth of the cases were diagnosed by examination of thick blood slides, a method which has advantages in that a long time can be taken in examining the slide and the slide can be kept for record. Another advantage is that different staining methods may be used. This method is also preferred by the patient because it is less painful.

Since trypanosomiasis is a very common disease in this area, any patient showing changes in the spinal fluid, such as increase in spinal fluid cell count and increased spinal fluid protein, may be given a tentative diagnosis of trypanosomiasis and 2.78 per cent of the cases were diagnosed by this method. In some of them trypanosomes were found in the spinal fluid, while in others trypanosomes were later seen in the thick blood film preparation. A spinal fluid cell count of 8 cells per c.mm. was considered normal. Any increase above this figure could be presumed to be due to trypanosomiasis.

Trypanosomiasis was more prevalent in the 20-29-year and 30-39-year age groups. The peak of the curve for the incidence of trypanosomiasis in 1943-1944 is in the 20-29-year age group; but in the group of patients diagnosed between

March, 1941, and May, 1943, the peak of the curve was in the 30-39-year age group. These are the two decades during which trypanosomiasis most affects the population, and it may be considered a disease of young adults. It is not a serious disease for the very young or very old. It affects the female population most during the child-bearing period and the male population most during the years when the earning capacity is greatest. It is also to be noted that these are the years when there is the greatest amount of exposure to the bites of tsetse-flies. It can be said that the distribution of cases is not what would be expected from a normal population curve. In most populations one would expect to have more cases in the group from 0-9 than in any other age group. There is a rise then in the curve from age 10 to age 39. The normal peak of the curve should be at the 0-9-year age group, whereas the peak of the trypanosomiasis curve is at the 20-29-year age group.

*Distribution of Cases by Age Groups*

YEARS	CASES
0-9	27
10-19	170
20-29	207
30-39	206
40-49	148
50-59	29
60-69	8
70-79	1

Two factors that may affect the curve are (1) a high birth rate thirty years ago, and (2) a recent decrease in the infant and child mortality. The population of this section of the country was probably greater at one time than it is now. The evidence for this belief is that there are many old town sites not occupied now. There is no evidence, however, that the birth rate was any higher thirty years ago than now. Regarding the second factor, there is no reason why infant mortality should have decreased, nor is there any evidence that it has. Living conditions in regard to sanitation and diet have not changed as far as can be ascertained.

Migration might affect the population curve, but there has been no general migration for the last fifty years. Another factor which might affect the curve from forty years onward is that from 1938 until 1940 the rice crops were very poor. The condition cannot be described as a famine, but the people were short of food and many deaths occurred in these years, possibly from trypanosomiasis, malaria or pneumonia. It may be said that the death rates were especially high during these years, which may account for the sudden drop in age distribution curve after the age of 40.

The high incidence of trypanosomiasis in the age group 20-39 is probably due to the fact that this group is more exposed to infection than the others.

A total of 516 cases of trypanosomiasis were diagnosed in Kissi country from January to November, 1944. An intensive effort was made from May until

September to examine all of the Kissi tribe and 401 cases were found during this period. The other cases were found on the two survey trips done in Kissi tribe or were cases that reported voluntarily for treatment.

The work in the Kissi tribe was carried on during the farming season, when it is very difficult to examine all the people because many sleep in their small farm kitchens and do not come into the towns at night. There were 18,950 persons examined during this diagnosis campaign and since 4,512 huts were counted, this gives 3.46 persons per hut. The Grigri and Poro Bush Schools were in session at that time and a considerable number of young people were kept there, so that few, if any, of this group of young people were diagnosed. Among the 18,950 persons examined from May to September in Kissi country, 401 positive cases were found, the rate of infection obtained from these figures being 2.56 per cent.

During the survey in the Buzzi tribe 107 cases were diagnosed and 14 more cases from this tribe came to the central diagnosis station for examination later, making a total of 121 cases. The survey in Buzzi country covered 13 of the larger towns, and the rate of infection found was 2.01 per cent. Many of the

*Distribution of Cases According to Tribes*

CASES	TRIBE
516	Kissi
121	Buzzi
92	Gbandi
24	Belle
24	Mandingo
8	Kpese
8	Mendi
2	Gio
1	Gola

patients of this tribe did not come in for treatment later, as they lived 40 to 70 miles from the treatment center. These people had had little education as to the value of treatment and medical work was not as popular there as in other tribes. During the first term of service treatment centers were located at Zorzor and Vassala, and it was here that the rate of infection was low. The Buzzi are a very large tribe and important economically to Liberia. During the year's work from December 20, 1943, until November 1, 1944, there has not been enough treatment to lessen the rates of infection to any great extent. The same rates probably prevail today as were found in February and March, 1944.

The Gbandi tribe has been important in trypanosomiasis work on account of its close proximity to the Kissi tribe. Much effort has been made to reduce the rate of infection in this tribe. A survey done in January and February, 1944, showed the incidence of trypanosomiasis to be 1.43 per cent. A systematic diagnosis was carried out in three of the Gbandi clans and 92 cases were found and treated. A survey made after the close of this work in September, 1944, showed that the rate of infection had been reduced to 0.45 per cent.

There were 24 cases of trypanosomiasis diagnosed in the Belle tribe. Many of these patients walked 50 to 75 miles for treatment. The rate of infection in this tribe was 2.75 per cent in January, 1944, the highest rate of any tribe examined in Liberia. While the tribe is small, it is important because of its close association with the Buzzi tribe. There has not been sufficient treatment to change the incidence of the disease.

The Mandingo tribe is a small tribe made up of traders who keep shops in native towns. They are great travelers, visit the coast towns and make long tours in Sierra Leone and French Guinea. It is difficult to get rates of infection among them, because there is no way to know how large the Mandingo population of the Western Province really is, but the rate seems to be relatively high. This tribe has probably had a great deal to do with the spread of the disease and has no doubt introduced it into new areas.

The Kpese tribe is a large tribe. Among them 720 persons were examined, with a rate of infection of 0.986 per cent. This is a coastal tribe and has little contact with interior tribes.

There is only one Mendi tribe in Liberia. This tribe was not visited during the present term of service. There were 8 cases that came in voluntarily for treatment. No facts are known about the rate of infection in this tribe at the present time.

*Grouping of Patients According to Estimated Stage of Disease*

		<i>per cent</i>
Early stage.....	122	15.0
Cases with enlargement of lymphatic glands.....	620	77.0
Cases with involvement of central nervous system.....	48	5.0
Terminal stage.....	6	0.8

The table showing the patients classified according to the estimated stage of the disease is interesting particularly as it shows that many of them are in an early stage. The classification is based entirely on a clinical estimate as to how far the disease has progressed. Most of the patients, 620, or 77 per cent, fell in the group with enlargement of the lymphatic glands, most of the diagnoses being made by aspiration of the material from the cervical lymph glands. This stage is marked either by a general glandular enlargement or by an enlargement of only the cervical group of glands. Trypanosomes are more easily found in relatively small glands. When the glands reach an enormous size and have a very soft center it is difficult to find trypanosomes. Occasionally a gland will be punctured when enlargement is due to tuberculosis. Tuberculosis does occur in these native tribes and the type is so severe that the patient does not usually live more than 90 days after the disease can be diagnosed. I have found 2 cases where I could demonstrate acid-fast organisms in the cervical lymph glands. In trypanosomiasis the stage characterized by enlargement of the glands lasts many months. The lymphatic response comes early and lasts until the nervous symptoms are far advanced.

The "early stage of disease" is difficult to define, but it can be said that all

early cases fall in this group. Nearly all these are difficult to diagnose because of the few trypanosomes in the peripheral blood stream and in the material aspirated from the glands. The glands are not markedly enlarged at this stage, usually about the size of a pea. There were 122 cases, or 15 per cent of all cases, in this group. Many of these patients are without symptoms, do not appear to be ill, and carry on their work as usual. A few complain of headache of a few months' duration.

In 48 cases, or 6 per cent, there was distinct involvement of the central nervous system. Some were insane or had difficulty in walking. The spinal fluid findings in these cases showed an increase in number of cells from approximately 250 cells upward and a spinal fluid protein of 67 centigrams per liter and upward. A large number of this group are relapse cases, while many others give a history of two or three years' illness. The prognosis of this group is always very bad, not only on account of the spinal fluid findings, but also because of the very poor nutritional state of the patients.

There were 6 terminal cases of trypanosomiasis, or 1.0 per cent of the group, nearly all showing advanced somnolence or insanity. In these, the spinal fluid protein is always high; all are poorly nourished and some develop bed sores. A large number of these cases die when they reach the sleepy stage. The prognosis for the insane patients is a little better than for the very sleepy ones.

*Symptoms of Pain*

SYMPTOMS	CASES	PER CENT
Headache.....	662	83.0
Backache.....	420	52.0
Abdominal pain.....	379	47.0
Pain in joints.....	433	54.0

Pain has long been known as one of the symptoms of trypanosomiasis. Since the clinical signs are more noticeable on examination, the tendency has been to overlook some of the symptoms which might be of assistance in diagnosing the disease. Trypanosomiasis has been a disease occurring chiefly in backward natives and language difficulties have often prevented the taking of accurate histories of the patient's symptoms. Pain is usually the first symptom of trypanosomiasis and is by far the most constant, usually occurring at all stages of the disease. In a series of 796 cases, 662, or 83 per cent, stated that they had headache for one year or more before the diagnosis was made. The headache is of a different type from that experienced from malaria or other tropical diseases. The descriptions of the type of headache vary a great deal, and pain is located in the top of the head. It is a dull type of pain and, while not too severe, is present at all times of the day or night when the patient is awake. Taking of food or hot or cold baths has no effect on the pain. It is very common for native patients to tie a cord tightly around the head in an attempt to alleviate the pain.

Pain in the joints is the next most common symptom of trypanosomiasis and in a series of 796 patients, 433, or 54 per cent, complained of this symptom. This symptom is not of much use in differential diagnosis because a large percentage



of patients suffering from yaws have pain in their joints. However, with this particular series of patients few had any clinical symptoms of yaws. The pains vary a great deal, some complaining of pain in their elbows and some of pains in their knees, or other joints.

Backache is a common symptom of trypanosomiasis and was present in 420 cases, or 52 per cent. This pain is usually in the lumbar region and the patients complain that the pain is constant and never quite disappears and that any exertion makes it worse. Backache is most often associated with headache and as the disease progresses this symptom usually becomes more marked. In the last stages of the disease the patients complain of excruciating backache.

Abdominal pain is a less common type noted in trypanosomiasis. In this series of cases, 379 patients, or 47 per cent, complained of abdominal pain. No specific area can be pointed out as a location of the pain and it varies greatly with different patients. Due to the prevalence of dysentery, some of these patients may have had pain from abdominal lesions not associated with trypanosomiasis. However, it may be said that since so many patients with trypanosomiasis complain of this symptom, it should be associated with the disease.

Many patients complain of two or more types of pain, particularly at the same time of headache, pain in the joints, and also in the abdomen.

*Duration of Illness from Onset of First Symptom*

LENGTH OF TIME	CASES	PER CENT
<i>months</i>		
0-5	82	10.3
6-11	47	5.7
Total for less than 1 year.....	129	16.0
12-17	397	49.8
18-23	3	0.4
Total for 1-2 years.....	400	50.2
24	196	24.6
No history of pain.....	71	9.2

The question listed on the history card is "Duration of illness from the first symptom". The first symptom always referred to pain, which was classified under one of the four headings shown in the table "symptoms of pain." The duration of illness from the first symptom referred to whichever one of the types of pain was first noted by the patient. Usually headache came first, but this was not always true as sometimes backache occurred without headache.

In 129 cases, or 16 per cent, pain was noticed for less than a year, and most of these had symptoms for less than 6 months. A large part of the cases diagnosed were in an area where there had been treatment for a period of 3½ years. Most of these cases were early and the laboratory findings made this clear, as

the spinal fluid cell counts were low. In these early cases headache was the most common type of pain. The onset of trypanosomiasis is so gradual that it is difficult for the patient to give exact dates, but pain is usually the first symptom noticed by the patient in the early stages of the disease.

The largest group was 400 cases, or 50.2 per cent, who gave a history of pain for 12 to 23 months before they were diagnosed. More patients set the date for the onset of their pain as one year ago and gave headache as their first symptom. From clinical observation I should judge that the incubation period of the disease is about 18 months. The greater part of this group were diagnosed by gland puncture. At this stage the trypanosomes can usually be found active in the material obtained by puncture of the cervical glands. This group of cases can still be considered as "early" and most of them can be successfully treated.

There were 196 cases, or 24.6 per cent, giving a history of 24 or more months. Most of our insane patients belonged to this group. Some of them can be diagnosed by blood slide or gland puncture, but most of them are diagnosed by spinal puncture. Some of this group were diagnosed clinically and later confirmed by laboratory findings. Some develop an ataxic gait similar to that seen in syphilis of the cord. The spinal fluid cell count is high in these cases and there is also an increase in the spinal fluid protein.

No history of pain was given by 71 patients, or 9.2 per cent. This group comprised 64 insane patients, from whom it was impossible to get any history, although some symptoms other than pain, such as sleepiness, could be had from relatives. However, there are a few patients who seem to have no pain at all and in these cases the somnolence usually begins early.

Pain is present in 16 per cent of the cases in the first year of the disease. Headache of long duration in a person in an endemic region of trypanosomiasis is strongly suggestive of this disease. The patients describe the type of headache in trypanosomiasis as of an entirely different kind from that of malaria or other diseases.

*Comparison of Sane and Insane*

	CASES	PER CENT
Sane.....	733	92.0
Insane.....	63	8.0

Insane cases are often encountered in the diagnosis of trypanosomiasis especially if the cases are of long standing. In general the prognosis is better for the insane cases than it is in the advanced sleepy cases.

Many of the deaths occurring among the insane trypanosomiasis cases are caused by starvation. The type of psychosis present varies a great deal, but is usually depression. Few of these cases are excited and have an increase in the store and flow of ideas. I have never seen a violently insane patient. These patients describe seeing lights and fires in the woods and hearing strange noises. It is common for this type of patient to run away and sleep in the bush, thus contracting pneumonia or other respiratory diseases, and many die as a result.

Others are in bad physical condition because they refuse to eat, starvation being common in this group of patients. Extreme depression is the most outstanding mental reaction in trypanosomiasis cases.

*Somnolence*

	CASES	PER CENT
Drowsiness in daytime.....	528	66.0
Restlessness at night.....	263	33.0
Aroused with difficulty.....	85	10.0
No symptoms of drowsiness.....	218	27.0

The common name "sleeping sickness" is derived from the tendency of trypanosomiasis patients to be drowsy especially in the daytime. This symptom has assumed a place all out of proportion to its importance in the disease, as it is present only in the more advanced cases. A large proportion of the early cases do not have this symptom at all.

In the series under discussion, 528 cases, or 66 per cent, had some drowsiness in the daytime. This does not mean that they spent most of their time sleeping, as most of this group still carried on their work normally. They complained, however, that when they sat down to rest in the daytime they immediately went to sleep. The onset of drowsiness is very gradual and patients usually do not notice this symptom as much as the relatives do. Restlessness at night was reported by 263, or 33 per cent of the cases, usually associated with pruritis of the skin on the chest. Some of the restless cases are sleepy in the daytime and others are not. Restlessness at night usually comes in the first year of the disease and as the disease progresses disappears altogether. The cases that have advanced somnolence are not restless at night.

The group of cases grouped under the heading "aroused with difficulty" are the far advanced patients who spend most of their time sleeping. The prognosis of this group is bad and about one-half of them die. There were 85 cases, or 10 per cent, in the group who had reached this stage.

*Duration of Drowsiness in the Daytime*

	CASES	PER CENT
From 0-5 months.....	113	14.0
From 6-11 months.....	71	8.0
Under 1 year, 0-11 months.....	184	23.0
From 12-17 months.....	305	38.0
From 18-23 months.....	2	0.2
12-23 months.....	307	38.2
From 24 months and over.....	87	10.0
No drowsiness at all.....	218	27.0

The symptom of drowsiness is not an early one in trypanosomiasis, and only 184 cases, or 23 per cent, noticed the symptom under one year. There were 307 cases who stated that they had been sleepy for over one year. There is a distinct correlation between the cases of headache and somnolence and the two curves run parallel. The beginning of lethargy corresponds very closely to the time when the spinal fluid count increases to 250 or more cells per cubic mm. of spinal fluid. At this point also the spinal fluid protein increases to 0.4 centigrams per liter of spinal fluid. The stage of sleepiness in the daytime, with or without restlessness at night, lasts about two years in an untreated patient before he enters the extreme lethargic state. As the patient's apathy increases, he becomes increasingly careless about his food and drink with the result that he usually contracts dysentery. Since the patients at this stage are less sensitive to cold due to their mental dullness, they sleep in damp, unsuitable places and may develop pneumonia. Intestinal disorders and respiratory diseases account for many of the deaths when the lethargy is advanced.

The long duration of this soporous stage gives evidence that the disease is of a chronic type and probably explains how a treatment campaign would reduce the incidence of the disease. It should be noted that 218 patients, or 27 per cent, showed no sign of drowsiness at any time during the course of the disease and that the group represents a collection of moderately advanced cases.

Five hundred and forty-eight patients, or 68 per cent, gave a history of fever in the last two weeks. Many patients had a normal temperature when the physical examination was made, but gave a history of fever nearly every evening. Irregularity is one of the characteristics of the temperature curve in trypanosomiasis. Often there is a rise in fever after the first or second treatment is given. Antrypol causes an increase in the patient's fever far more often than tryparsamide. Since most patients continue with their normal work for months after trypanosomes can be found in the cervical lymph glands, the temperature in most cases must not be high. This shows again that, in the region investigated, the disease is of the chronic type.

*Prognosis*

	CASES	PER CENT
Good.....	103	13.0
Fair.....	512	64.0
Poor.....	181	22.0

In noting the prognosis of each patient, the plan was to classify the patients not only by laboratory findings, but also to take into account age and nutrition and several other factors that go to make a bad risk for treatment. The very young and very old do not respond well to treatment. Many writers have pointed out that other co-existing diseases, such as schistosomiasis and ankylostomiasis, make the prognosis much worse. Pregnancy makes prognosis more grave in trypanosomiasis and causes abortion in 7 per cent of the cases, according to Kellersberger (1933). The presence of any respiratory disease is always

serious and smallpox has been encountered in both terms of service as existing in epidemic form in the area where the work was done. The nutrition of the patient is also very important in this disease. The group rated "good" was made up largely of well-nourished males with no other disease present. It consisted of 103 cases, or 13 per cent.

The largest group were rated "fair", with 512 cases or 64 per cent. There was at least one unfavorable factor in each of these cases.

The group rated "poor" comprised 181 cases or 22 per cent. Two or more adverse factors made this group a bad risk.

*Glandular Enlargement*

	CASES	PER CENT
<b>Cervical</b>		
Size of a pea.....	128	16.0
Size of a marble.....	458	57.0
Size of a pigeon egg.....	207	26.0
No enlargement.....	3	0.3
<b>Epitrochlear</b>		
Size of a pea.....	334	42.0
Size of a marble.....	170	21.0
Size of a pigeon egg.....	18	2.0
No enlargement.....	274	34.0
<b>Inguinal</b>		
Size of a pea.....	114	14.0
Size of a marble.....	329	41.0
Size of a pigeon egg.....	161	20.0
No enlargement.....	192	24.0

Enlargement of the cervical lymph glands has long been known to be a symptom of trypanosomiasis and, in a series of 796 cases, 793, or 99.7 per cent, showed enlargement of the cervical lymph glands. In more than one-half of these the glands were the size of a marble, and in 26 per cent of the cases they were as large as a pigeon's egg. There were only 0.3 per cent of the cases which showed no enlargement at all. Glands the size of a marble were the most common type found in the cervical group.

The epitrochlear glands usually show some enlargement in trypanosomiasis, and the most common size for these glands found was that of a pea. The epitrochlear glands were seldom enlarged to a very great size, but showed some enlargement in 66 per cent of the cases. Occasionally, they are large enough to puncture. Cuts often occur on the hands of the patients, so that some of these swollen epitrochlear glands may be due to other infections and not to trypanosomiasis.

In 604 cases, or 75 per cent, there was an enlargement of the inguinal lymph glands. There are many conditions that cause an enlargement of these glands, such as lesions of yaws on the feet, tropical ulcer, cuts and bruises. Another

cause for enlargement of these glands is syphilis. However, swelling of the inguinal glands is a very common sign in trypanosomiasis.

Trypanosomiasis is a disease in which there is a general glandular enlargement involving nearly all of the lymphatics of the body. Swelling is seldom localized in any one group of glands. The symptoms of glandular enlargement come on early in the disease and remain until the terminal stage appears when the glands become smaller and soft. When treatment is started and the trypanosomes disappear from the blood, the glands become firm and hard and resolution after the recovery of the patient does not take place for from 4 to 6 months. The response of the glandular tissue is one of the first changes that take place when the body reacts to the invasion by trypanosomes. The resolution of lymphatic glandular tissue is one of the last signs of trypanosomiasis to disappear when the patient is fully recovered.

*Splenic Enlargement*

	CASES	PER CENT
Palpable.....	239	30.0
Level of umbilicus.....	15	2.0
Below umbilicus.....	1	0.1
No enlargement.....	541	66.0

Splenic enlargement was found in 30 per cent of the cases of trypanosomiasis. It is well known that both malaria and schistosomiasis are prevalent in this section of Africa and that enlargement of the spleen is common in these diseases. An enlargement of the spleen occurs most frequently in children from 1-12 years of age. In the present series of cases there were only 27 cases, or 3 per cent, in the age group from 0-9 years. The age group which contains the greatest number of patients is from 20-39 years, and normally adults do not have enlarged spleens in this section. The splenic enlargement in trypanosomiasis ordinarily is one that is just palpable.

There were 239 cases, or 30 per cent, in which the spleen was palpable and only in about 2.1 per cent of the cases was the spleen greatly enlarged.

After treatment has begun, the enlargement of the spleen due to trypanosomiasis quickly disappears and the response of the spleen to the invasion of trypanosomes seems to run parallel to that of the lymphatic system.

Nutrition is very important because this one factor has much to do with the patient's recovery, being often the determining factor. Starving patients show little improvement, no matter how much of the drug they receive. Nutrition gives an index as to how far the case has progressed and different stages of starvation are seen in cases involving the central nervous system, because the patient refuses to swallow his food. Malnutrition appears early in trypanosomiasis and one of the first symptoms noticed is that the patient begins to lose weight and acquires a peculiar gray line on the margin of the gums.

Since 459 cases, or 57 per cent, were in the group classified "fair" for nutrition, this suggests that most of the patients were early cases. One confusing factor

in diagnosing malnutrition in trypanosomiasis is the fact that early cases often show puffiness of the face. This is not a true oedema and will not pit on pressure. This symptom comes early, is a part of the syndrome of dullness, apathy and lethargy, and does not disappear until just before death. Since the entire population of the area where the survey was made consumes very little protein, it is difficult to determine what the normal standard of diet is. Rice, greens and palm oil make up the principal food of the people. The tribes are pure negroid. The normal color of their skin is black with an oily appearance, and when they are ill this changes to an ash or gray. Malaria, anemia and syphilis and many other diseases will produce a change in color and appearance. Among the trypanosomiasis cases, 291, or 39 per cent, showed this gray color. There is a close correlation between this group of patients with gray color and those who are poorly nourished.

*Various Symptoms*

	CASES	PER CENT
Nutrition		
Good.....	108	13.0
Fair.....	459	57.0
Poor.....	229	28.0
Color of Skin		
Good.....	505	61.0
Gray.....	291	39.0
Pinching		
Firm.....	477	59.0
Flabby.....	319	41.0

Most texts of tropical medicine describe skin lesions occurring in trypanosomiasis cases. In the present group of 796 cases, there were no skin lesions characteristic of the disease. There were some marks on the skin caused by scratches on the chest due to the severe pruritis that occurs during the first year of the disease. A fungus infection occurring on the chest was noticed in many cases, but no skin lesion was present, so far as could be made out, which was typical of trypanosomiasis. The changes in the appearance of the skin described by Kellersberger were noted. This loss of healthy appearance of the skin and extreme dryness with pruritis were commonly seen.

The next sign noted was the condition of the nutrition of the patient elicited by pinching, noting the condition of muscular tone. There was a flabbiness found in 319 cases or 41 per cent. Early in trypanosomiasis this loss of muscular tone is seen and accounts for the shuffling gait and apathy in all the movements of the patient. The flabbiness of the muscles of the patient is nearly always associated with a gray skin color and very poor nutrition. The reflexes of this group are not always the same, some being absent and some exaggerated.

*Other Diseases Present in Trypanosomiasis Cases*

DISEASE	CASES	PER CENT
Yaws.....	147	18.0
Malaria.....	174	21.0
Tropical ulcer.....	11	1.0
Dysentery.....	6	0.7
Leprosy.....	2	0.2
Other skin diseases.....	70	8.0
Elephantiasis.....	4	0.5
No other disease present.....	536	67.0

Yaws is a disease very common in all parts of Liberia. The crowding of many natives in one house is one of the factors which helps spread the disease and the greater the house population the greater the incidence of yaws. This fact has been brought out in investigation in Sierra Leone. Among the present group of trypanosomiasis, 147, or 18 per cent, had yaws. However, it cannot be considered a serious complication. Treatment with tryparsamide will cure most cases of yaws. There is no contraindication to giving bismuth preparation for yaws at the same time the patient is receiving tryparsamide treatment for trypanosomiasis. Most of the cases of yaws are of the latent and non-infectious type.

Malaria was present in 174 cases, or 21 per cent. It is usually not a serious complication and the cases clear up quickly on quinine or atabrine treatment. All cases of malaria in this series were diagnosed by a thick blood film. A large number of cases were in the very young or very old age groups.

Tropical ulcer (tropical sloughing phagoedema) was present in 11 cases and is a serious complication of trypanosomiasis. The reason for this is that the nutrition of such patients is always bad. The arsenic in the form of tryparsamide as a rule does not produce any beneficial effect. Natives have an idea that an ulcer case should not eat fish or meat and this lack of protein in the diet makes the condition much worse. As a result anemia is present in a large number of ulcer cases.

Amoebic dysentery was present in 6 cases and is a serious complication because these patients are acutely ill.

Leprosy was present in 2 cases. These cases usually respond well to treatment and their leprosy seems to improve while they are on tryparsamide treatment.

Elephantiasis was present in 4 cases only and the patients had marked swelling of the feet. It is fairly common to find microfilariae in the blood film or in the material aspirated from the cervical lymph glands, 32 cases being found during the year. However, in these cases where microfilariae were found, no swelling of any part of the body could be observed, and the cases were symptomless.

Under the heading of other skin diseases we find a large group of skin conditions. The most common of these was crawl-crawl and the next in importance was the group of fungus diseases of the ringworm variety. Scabies was also present in a considerable number of cases and insect bites of all kinds were also seen.



*Nervous Reactions in Trypanosomiasis*

	CASES	PER CENT
Romberg's sign.....	370	46.2
Parkinson's syndrome.....	23	2.8
Babinski's sign.....	46	5.7
Tremor of hands.....	278	34.9
Tremor of tongue.....	264	33.1
Kerendal's sign.....	80	10.0

There are many nervous manifestations of trypanosomiasis, some of which are present early in the disease, while others occur in the later stages.

Romberg's sign was noted in the greatest number of cases and was present in 370, or 46 per cent. This reaction can be found in relatively early cases of the disease and is very marked in the late stages. It is usually present when there is any increase of the spinal fluid protein and also when there are more than 50 cells per cubic millimeter of spinal fluid. It is true that this sign is also positive in syphilis of the central nervous system, but since there are so few cases of this type of syphilis in the native population, this is not a serious problem in differential diagnosis.

The Babinski reflex, the extension of the toes instead of flexion on stimulation of the sole of the foot, which indicates a lesion of the pyramidal tract, was found in 46 cases of trypanosomiasis, or only about 5.7 per cent of the whole group. It was present only in far advanced cases.

Parkinson's syndrome is a condition characterized by muscular rigidity, an immobile face, and tremor which tends to disappear on volitional movements. It was seen in 23 cases, or 2.8 per cent of the cases. These cases were of two types. Eighteen developed the tremor and associated nervous conditions after they reached the stage of advanced trypanosomiasis. The other 6 cases were patients who had had Parkinson's syndrome over a period of years and developed trypanosomiasis late in life. If the syndrome is a result of far advanced trypanosomiasis, the prognosis is very grave. This syndrome occurs in a small percentage of trypanosomiasis cases and is a result of trypanosome invasion of the central nervous system.

Kerendal's sign, which is a hyperaesthesia of the skin with slightly delayed sensation of pain, was seen in 80 cases, or 10.0 per cent of all patients. It is very difficult to get definite information on this subject because of language difficulties. The sign is useful in some cases, but the difficulties in obtaining a correct answer from native people on a question of relative amount of pain is very close to impossible. This sign has probably been given undue emphasis as a diagnostic help in trypanosomiasis.

The second most common nervous symptom found was tremor of the hands, with 278 patients, or 34.9 per cent, having this symptom. The tremor appears early in the disease and becomes gradually worse as the spinal fluid changes progress. Closely associated with this is the tremor of the tongue which occurred in 264 cases, or 33.1 per cent.

The three outstanding nervous signs seen early in trypanosomiasis and remaining fairly constant are Romberg's sign, tremor of the hands and tremor of the tongue.

*Patellar Reflexes*

	CASES	PER CENT
Knee jerk normal.....	94	11.8
Exaggerated.....	559	68.59
Absent.....	143	- 17.96

The most common reaction in the moderately advanced cases was an exaggerated knee jerk. In very early cases the knee jerk was normal and those having an absence of this reflex were nearly all far-advanced cases.

*Pupillary Reflexes*

	CASES	PER CENT
Normal.....	754	95.97
Unequal.....	37	4.66
Fixed.....	32	4.02

A large per cent of the cases had pupils that reacted normally to light and distance. There were a very few far-advanced cases who had fixed and unequal pupils.

*Pulse Rates*

PULSE	CASES
50-59	10
60-69	108
70-79	152
80-89	180
90-100	118
100-109	190
110-119	18
120-129	5
over 130	0
Unknown	14

Most cases of trypanosomiasis do not have a high pulse rate and the pulse was normal in many cases. The disease is of a chronic type with a slow onset. Since very little fever is present, there is not much change in the pulse rate early in the disease. The pulse does increase when the patient has fever, but usually subsides when the temperature returns to normal. As a general rule the pulse was relatively low in relation to the temperature and the quality of the pulse was good in this group of patients. There were only 23 patients with a pulse of more than 110. During the terminal stage of the disease the pulse usually remains

between 100 and 120 and the quality of the pulse is very poor. A saline and glucose solution given intravenously improves the pulse markedly, but is only of temporary value. The pulse is not of much aid to diagnosis as there is nothing particularly characteristic in its rate or quality.

#### Temperature

	CASES
Less than 98.6°.....	164
Exactly 98.6°.....	171
98.7-99.8.....	273
100-101.8.....	172
102-103.8.....	1
105.....	1
Unknown.....	14

Irregularity of temperature is one of the most characteristic things about trypanosomiasis. There are long periods when the patient's temperature is normal, then he will run a fever of 102° or higher for a few days, after which his temperature returns to normal. There were 171 cases, or 20 per cent, who had normal temperatures, when examined. In the greater number of patients the temperature was from 98.7° to 100°. The temperature tends to increase more than the pulse rate, but very little is characteristic about the temperature curve of trypanosomiasis. Even in the terminal cases of the disease it does not go above 103° and some of the cases do not have more than 100° at the time of death.

#### Spinal Fluid Protein

(428 patients considered in this group)

CENTIGRAMS PER LITER	CASES
0.22	248
0.40	132
0.50	1
0.56	11
0.67	18
0.71	3
0.78	13
0.85	2

A spinal puncture was done on 428 cases and the amount of protein determined by the following method:

#### *Determination of the Amount of Protein in Spinal Fluid by Means of the Sicard-Cantaloube Tube*

1. Collect 4 cc. of spinal fluid in the Sicard-Cantaloube Tube. The mark on the tube is at 4 cc.; if too much fluid is collected in the tube, remove with pipette.
2. Heat the tube above alcohol flame to 80-90°C.; at that point bubbles begin to appear.
3. Immediately after, drop in 12 drops of a 33½ per cent solution of trichloroacetic acid.
4. Let the tube stand for 5 minutes.
5. Reverse the tube (well stoppered with rubber) two or three times.

6. Let the tube remain supported in a strictly vertical position for 5 hours, the optimum lapse of time.

7. This time having passed, read the highest mark on the graduated scale.

Note: In normal spinal fluid the protein is 0.22 per cent. The unit in which the amount of protein is expressed by the Sicard-Cantaloube method is that a normal fluid will give 0.22 centigrams to the liter of spinal fluid. Any increase above this amount can be expressed directly in centigrams by reading from the tube.

A total of 248 cases, or 58 per cent, showed a normal amount of spinal fluid protein, indicating that a large proportion of the cases seen were early cases. It also shows that the protein response is later in appearing than the increase in the spinal fluid cell count. The relapsed cases nearly all showed a spinal fluid protein reading of 0.40 centigrams per liter or more. The finding was more consistent than a uniformly high spinal fluid cell count. The cases showing advanced involvement of the central nervous system showed a spinal fluid protein reading of 0.56 centigrams per liter or higher. Practically all the cases die when the spinal fluid protein reading is 0.85 centigrams per liter. Two cases reached the point of 1.0 centigrams per liter, but both died a few days later. The cases having a reading of 0.67 centigrams per liter are border line cases and have about an equal chance for recovery or death. A spinal fluid protein determination gives an indication of how far advanced the disease is at the beginning of treatment. The spinal fluid protein remains high for some time after the patient has improved clinically; it should remain normal for 6 months or more after the treatment before the patient can be considered well. In some respects the findings run parallel to the spinal fluid cell count findings, but the determination of the amount of protein is worth doing in all cases for the additional information it gives. An examination of a case of trypanosomiasis is not complete unless the spinal fluid protein has been determined.

*Spinal Fluid Cell Count*  
(421 cases considered in this group)

CELS	CASES
0-9	21
10-29	240
30-49	118
50-69	26
70-89	22
90-109	6
110-299	21
300-399	2
400-499	2
500-599	0
600 or more	3

There is a wide range in spinal fluid cell counts with trypanosomiasis in this series of 421 cases. All patients with a cell count under 100 cells per millimeter showed no signs of the nervous symptoms of the disease.

jority of the cases give a cell count of 10-49 cells per cubic millimeter. Another phenomenon in the study of spinal fluid changes in trypanosomiasis is that the cellular response comes first and increase in the spinal protein comes later. The moderately advanced cases show many variations, but the greatest number have 30-49 cells per cubic millimeter. There were only 82 cases who were above this mark and they are among the very late cases. When treatment is begun the cell count usually stays at the same level for a time and then gradually decreases. In cases which are not treated the cell count goes up from 500 to 1,000 cells and then seems to decrease or stay at that level. When this point is reached the spinal fluid protein continues to rise until death occurs. The spinal fluid cell count is of the utmost importance in classifying patients and prescribing proper treatment for them. The cell count during the treatment period is of great value as an indication of the progress of the patient. It is the most useful way of finding out whether the patient has recovered and is not likely to have a relapse. In an area where trypanosomiasis is endemic, a patient who has an increase in the spinal fluid cell count with an associated increased spinal fluid protein probably has trypanosomiasis, if other diseases such as syphilis can be ruled out.

*Spinal Fluid Protein*

SPINAL FLUID CELL COUNTS	0.22	0.40	0.50	0.56	0.67	0.71	0.78	0.85	UNKNOWN	TOTAL	PERCENTAGE NORMAL FLUID PRO- TEIN COUNT	PERCENTAGE PATHOLOGICAL PRO- TEIN COUNT
0-9	13	6		2						21	61	39
10-29	142	49			3	2	4			200	71	29
30-49	65	44	1	3	3		2			118	55	45
50-69	12	11		1				1	1	26	46	54
70-89	9	9		1	3					22	40	60
90-109	0	3			2		1			6	0	100
110-299	2	7		2	6	1	3			21	9	91
300-399				1			1			2		100
400-499		1		1						2		100
500-599												
600-							2	1		3		100
Total.....	243	130	1	11	17	3	13	2	1	421		

The table showing the relation of the spinal fluid cell count and the spinal fluid protein is interesting. A normal spinal fluid cell count is 9 cells or less per cubic millimeter of spinal fluid and any cell count above 9 cells is pathological.

The reading of 0.22 centigrams per liter of protein in the spinal fluid is normal and all above this reading pathological. It has long been known that the characteristic spinal fluid changes in trypanosomiasis are an increase in the cell count and an increase of the protein. This table shows in general that patients whose cell counts were low also had a low spinal fluid protein and those with high spinal fluid cell count also had a high spinal fluid protein. This tends to show that in the progress of the disease from a very early stage to a far advanced

one involving the central nervous system there is an increase in both cell count and the amount of protein in the spinal fluid.

The first changes observed in the spinal fluid in trypanosomiasis is an increase in the number of cells. Following this change by several months there is usually an increase in the spinal fluid protein.

In the first group of patients who had a cell count of 0-9 cells per cubic millimeter, there were 61 per cent who had a normal spinal fluid protein (0.22 centigrams per liter). Theoretically this group should have been 100 per cent but there were 39 per cent who showed some pathology in the spinal fluid protein.

As shown on the second line of the table in the group of cases having a cell count of from 10-29 cells, there were 142 patients or 71 per cent who showed an increase in the spinal fluid cell count but who had a normal spinal fluid protein. In comparison with this figure there were only 29 who showed pathological spinal fluid protein. Nearly one-half the cases in the series fall into this group and it is significant because the sample is large.

In the third line of the table, in the group of cases having from 30-49 cells per cubic millimeter in spinal fluid, there were 65 cases or 55 per cent who showed an increase in the cell count but a normal spinal fluid protein. In this same group, however, there were 45 per cent who showed a pathological spinal fluid protein.

In the group of patients who showed 50-59 cells per cubic millimeter of spinal fluid, there were 12 cases or 46 per cent with an increase in cell count but a normal spinal fluid protein. In this same series there were 54 per cent with pathological spinal fluid protein.

In the next group of patients having from 70-89 cells per cubic millimeter of spinal fluid, there were 9 cases or 40 per cent who showed an increase in the spinal fluid cell count but normal spinal fluid protein. There were 60 per cent of this series who had a pathological spinal protein.

In the remainder of this table there was pathology in all the spinal fluid proteins with the exception of 2 cases. When the cell count reached 90 cells per cubic millimeter, there was a rise in the spinal fluid protein in nearly all cases. There was a lag in the appearance of spinal fluid protein pathology as compared to cellular pathology.

In comparing the percentages of normal spinal fluid proteins and pathological spinal fluid proteins we find that protein response is slower in appearing in the disease than an increase in the spinal fluid cell count, and also the spinal fluid cell found is a more sensitive indicator in determining pathology of the spinal fluid in trypanosomiasis.

The purpose of this table was to ascertain what the average spinal fluid cell count was at each stage of the disease.

There was an average of 35.94 cells per cubic millimeter of spinal fluid in Group A. There were only 21 cases in this whole series that showed normal spinal fluid cell count. There were 77 cases in the first group, which is a fair sample.

The largest number of cases appear in Group B. The average number of cells in this group was 42.1 per cubic millimeter of spinal fluid. This stage of the

disease continues for a very long time in untreated cases and the number of cells in this stage as compared with the first stage shows only a very small rise.

*Spinal Fluid Cell Counts with Their Relation to the Four Stages of the Disease*

FLUID CELL COUNT	ESTIMATED STAGE OF THE DISEASE				
	<i>A</i> Stage of invasion	<i>B</i> Enlargement of lymphatics	<i>C</i> Involvement of central nervous system	<i>D</i> Terminal cases	Total
0-9	5	14	2		21
10-29	39	149	12		200
30-49	21	89	7	1	118
50-69	6	16	4		26
70-89	3	16	3		22
90-110		3	3		6
110-209	3	15	2	1	21
300-399		2			2
400-499			2		2
500-599					
600+			2	1	3
Total .....	77	304	37	3	421
Avg. no. of cells....	35.94	42.1	82.24	103.0	

The number of cases in Group C is small, with only 37 patients. The average number of cells in this group was 82.24 per cubic millimeter of spinal fluid, which is almost double that of the Group B.

In Group D there were only 3 cases with a spinal fluid cell count average of 103 cells per cubic millimeter. The cases were very far advanced and 2 of them died. There is a gradual rise of the number of cells in the spinal fluid during the progress of the disease. The greatest increase in the number of cells is in the later stages as shown in Groups C and D.

*Spinal Fluid Protein Determination, in Relation to the Four Stages of the Disease*

SPINAL FLUID PROTEIN	<i>A</i> STAGE OF INVASION	<i>B</i> ENLARGEMENT OF LYMPHATICS	<i>C</i> INVOLVEMENT OF CENTRAL NERVOUS SYSTEM	<i>D</i> TERMINAL CASES	TOTAL
0.22	55	180	13		248
0.40	18	101	13		132
0.50		1			1
0.56		9	2		11
0.67	2	12	4		18
0.71	1	2			3
0.78	2	5	3	3	13
0.85			2		2
Total. . . . .	78	310	37	3	
Average number of cells.	0.29	0.32	0.42	0.78	

The above table is of value because the measure of spinal fluid protein gives some indication as to the amount of destruction that has taken place in the brain and spinal cord. As has been shown before, the protein response is slow in appearing, but also indicates a longstanding infection and a certain amount of destruction. Many patients having a very high protein content do not respond to treatment and may die. All the patients with spinal fluid protein readings of 0.78 and 0.85 died.

In Group A there were 78 patients and their average spinal fluid protein was 0.29 centigrams per liter of spinal fluid. The normal spinal fluid protein is 0.22 centigrams per liter. It may be seen that this early group of patients has only a slight increase of spinal fluid protein.

In Group B there were 310 patients with an average increase in the spinal fluid of 0.32 centigrams per liter. Even at this stage the rise in spinal fluid protein is not marked.

In Group C there were 37 patients with an average of 0.42 centigrams per liter of spinal fluid. At this stage of the disease the rise in protein is marked.

In Group D there is an average of 0.78 centigrams per liter of spinal fluid. None of this group showed response to treatment and died soon after treatment was begun.

This table shows that as the disease progresses from the stage of invasion to the terminal stage there is a gradual rise of the spinal fluid protein.

*Comparison of New and Relapsed Cases as to Spinal Fluid Cell Counts and Spinal Fluid Protein*

	SPINAL FLUID PROTEIN								Total
	0.22	0.40	0.50	0.56	0.67	0.71	0.78	0.85	
Relapse cases.....	39 41%	34 36%		3 3%	5 5%		3 3%		94
New cases.....	209 50%	98 28%	1	8 2%	13 3%	3 2%	10 3%	2	344

	SPINAL FLUID CELL COUNT											Total
	0-9	10-29	30-49	50-69	70-89	90-109	110-299	300-399	400-499	500-599	600+	
Relapse cases.....	4 4%	32 39%	24 29%	4 4%	5 6%	6 7%	4 4%				1	81
New cases.....	17 4%	168 48%	94 21%	22 6%	17 4%		17 4%	2	2 3%		2	345

The point of interest in these two tables is that only 41 per cent of the patients in the relapse group had a normal spinal fluid protein, while 66 per cent of the new cases show no change in the spinal protein. This was not the case in the spinal fluid cell count, as both new and relapsed cases show only 4 per cent having a normal spinal fluid cell count. Again this table shows that the spinal fluid cell count increases earlier in the disease than the spinal fluid protein.



*Miscellaneous symptoms.*—Sexual impotency is a common complaint in male trypanosomiasis cases and sometimes this is the first symptom of the disease. Many of these cases do not have headache. It is usually associated with malnutrition. The condition nearly always clears up after the fourth or fifth treatment.

Another symptom often seen in fatal cases is effusion into the joints and this condition has been seen in 5 fatal cases. All the joints of the body are involved and it causes the patient a great deal of pain when he is moved. The condition comes on suddenly and is usually associated with high fever. Most of these patients died on the fourth or fifth day after the effusion of the joints developed.

#### GENERAL REMARKS ON TREATMENT

The following treatments were given during the last term of service from December, 1943, to November, 1944.

NUMBER OF PATIENTS	NUMBER OF TREATMENTS
48	0
19	1
15	2
17	3
11	4
18	5
14	6
44	7
106	8
50	9
454	10

The total number of single treatments given was 6,564. A total of 668 patients, or 83.94 per cent, received 6 or more treatments; 80 cases, or 10.1 per cent, received from 1 to 5 treatments; and 48 cases, or 6.03 per cent, were diagnosed on the survey trip but did not report for treatment at all. There were 610 cases, or 76.63 per cent, who received 8 or more treatments.

The reason why a larger percentage of cases did not finish treatment was the fact that the survey tours were long and the diagnoses were made on these trips. In many instances the patient would have had to walk from 3 to 10 days in order to reach a treatment center. In the Kissi section, where intensive work was done, 92.48 per cent of the patients completed treatment.

Two drugs were used in the treatment of trypanosomiasis, antrypol, manufactured by May and Baker, Limited, in England, and tryparsamide, made in the United States.

One plan for treatment used tryparsamide only, and the other three plans used a combination of antrypol and tryparsamide.

When tryparsamide was used alone it was given in 10 doses spaced from 5 to 7 days. The first dose consisted of 1.0 gram in 5 cc. of freshly distilled water and all subsequent doses were each of 2.0 grams in 10 cc. of distilled water. These doses were given to all the adult patients weighing 100 pounds or more, but were reduced for children. The total average dose given to one individual was 19

grams. This method has the advantage that it is useful at all stages of the disease and that a very small number of reactions occur. In introducing treatment into a new and fearful group of patients, tryparsamide is the drug of choice.

The second plan of treatment is first a dose of antrypol followed by 9 injections of tryparsamide. One gram of antrypol is given for the first dose in 10 cc. of distilled water. The second dose consists of 1.0 gram of tryparsamide, and all following doses from 3 to 10 inclusive are 2.0 gram doses of tryparsamide. The total amount of drug used in this plan is 1.0 gram of antrypol and 17 grams of tryparsamide. Fainting and vomiting occurred in a small number of antrypol cases. The palms of the hands and soles of the feet remain tender from 1 to 4 days after treatment. The patients say the drug makes their "head turn", meaning dizziness, in a large number of cases. The greatest advantages of the use of antrypol are that it does not produce eye symptoms and that it is a good public health procedure because trypanosomes disappear more quickly from the circulatory system than when tryparsamide is used alone. After one injection of antrypol it is practically impossible to demonstrate trypanosomes in the peripheral blood, whereas trypanosomes can sometimes be found after 1 or 2 treatments of tryparsamide. After antrypol is administered it is practically impossible for flies to pick up an infection of trypanosomes. All the patients treated in my first term of service, 1941-1943, with the exception of one group, were treated by these methods.

One group of patients were treated in 1944 in the town of Babahun in the Gbandi section, with 3 injections of antrypol, 1.0 gram each, at 5-day intervals and then 5 doses of tryparsamide, with 1.0 gram for the first dose and 2.0 grams for the other doses. This method has been used in other trypanosomiasis-treating campaigns, especially in Nigeria. A series of 686 cases were treated by this method. After the first or second dose of tryparsamide, 9 out of this group, or 1.3 per cent, developed severe arsenic dermatitis. In a series of 1928 other cases studied from 6 to 20 months after treatment with tryparsamide, only .02 per cent developed a severe dermatitis. It appears that either antrypol when given in 3 consecutive doses produces a severe skin reaction or it produces a sensitization so that the arsenic in the tryparsamide gives the dermatitis. There were 12 cases that developed a severe oedema of the feet with albumen and casts in their urine. All these cases cleared up after from 2 to 3 weeks' treatment. There were 6 deaths in this group during the time of treatment; 2 died of pneumonia, 2 with chills and fever, probably malaria, and 2 from trypanosomiasis.

During the 1943-1944 term of service, for the American Foundation for Tropical Medicine, Inc., a different combination of treatment was used. This plan is shown in upper table on page 44.

No severe reaction causing dermatitis or oedema of lower extremities with nephritis was observed. It is too early to check the results of this method of treatment. By using this plan the maximum amount of antrypol can be used with the least toxic effects to the patient.

During the 1943-1944 survey it was also possible to examine 2,764 cases which had been treated previously. These showed about 4.0 per cent relapses.

Those treated with tryparsamide alone and those treated with a combination of antrypol and tryparsamide showed about the same number of relapses.

DOSE	AMOUNT	DRUG USED
	<i>grams</i>	
1st	1.0	antrypol
2d	1.0	tryparsamide
3d	2.0	tryparsamide
4th	1.0	antrypol
5th	2.0	tryparsamide
6th	2.0	tryparsamide
7th	2.0	tryparsamide
8th	2.0	tryparsamide
9th	2.0	tryparsamide
10th	2.0	tryparsamide

#### REPORT OF DEATHS

During the period from December 20, 1943, to November 1, 1944, 796 patients were diagnosed and of these 14 died, giving a death rate in this group of 2 per cent. Other deaths will probably occur due to trypanosomiasis during the next year in this group but there seems to be no way to find out the facts regarding these patients. It is also known from previous work that approximately 4 per cent of these cases will relapse. Death will probably follow in many of these relapse cases.

However, an analysis will be made here of the facts that are known about these 14 deaths. There were 10 males and 4 females; 9 were of the Kissi tribe, 4 of the Buzzi tribe and one of the Gbandi tribe. One case had relapsed after he had treatment two years ago. All the others were new cases. As to the method of diagnosis, 2 were made by examination of a preparation of thick blood smear, 3 by clinical diagnosis, and 9 were made by examination of material obtained by a puncture of the cervical lymph glands. The distribution of the cases by age group is interesting:

AGE	CASES
<i>years</i>	
0-9	0
10-19	5
20-29	3
30-39	4
40-49	1
50 and over	1

It appears that the deaths from trypanosomiasis do not occur in the very young or the very old. Most of the deaths are in the 20-39 age group. In the original 796 cases this was the age group in which there were most cases. Many of the fatal cases were far advanced and some died soon after treatment was started. Three of these cases did not receive any treatment and four had only one treat-

ment; two cases had three treatments and 2 had four treatments. Only three fatal cases received a course of eight treatments. In these three cases death occurred soon after the completion of treatment.

DIAGNOSIS DATE	DEATH DATE	APPROXIMATE NUMBER OF MONTHS AFTER DIAGNOSIS PATIENT DIED
Jan. 30, 1944	Apr. 1, 1944	2
Feb. 6	Apr. 7	2
Feb. 6	Apr. 8	2
Apr. 6	Apr. 10	2
Apr. 13	July 10	3
Apr. 21	Aug. 10	4
May 1	Aug. 11	3
May 1	May 12	$\frac{1}{2}$
June 27	Aug. 22	2
July 20	July 29	$\frac{1}{2}$
July 21	July 30	$\frac{1}{2}$
July 22	Oct. 26	4
July 23	Sept. 29	2
Aug. 6	Aug. 26	$\frac{1}{2}$

4 cases died  $\frac{1}{2}$  month after diagnosis

6 cases died 2 months after diagnosis

3 cases died 3 months after diagnosis

1 case died 4 months after diagnosis

Most of these cases died soon after treatment was given. Those who received no treatment were cases diagnosed during one of the survey trips. One of these cases was so far advanced that he could not walk and was carried in. Three of this group were insane and a history had to be taken from a relative. In carefully taking a history of these cases the following facts were found: There were only two cases who had been ill for less than one year. Six cases gave a history of one year duration, four gave a history of two years duration, one case of three years duration and one case of four years duration. The neurological findings of these cases vary greatly. There were eight with a positive Romberg sign.

Consideration of spinal fluid cell count and spinal fluid protein in the 7 fatal cases.—There were 14 deaths in the series of 796 cases but only 7 of this group had a spinal puncture done, so that we have laboratory findings on only 7 cases.

The spinal fluid cell count showed that in only one case was the count extremely high.

CELLS PER CU. MM.	CASES
410	1
50	1
35	2
25	1
16	1
15	1

Most of the cases showed only a slight increase in the number of cells present in the spinal fluid. Some with low cell counts probably developed some inter-current disease which was partly responsible for the deaths.

The amount of protein present in the spinal fluid is interesting.

CASES	PROTEIN ESTIMATION
1	0.85
1	0.78
2	0.40
3	0.22

Two of these cases had a very high protein estimation and were very late cases. Death occurred in both very soon after the diagnosis was made. There were two moderately advanced cases with spinal fluid protein of 0.40 centigrams per liter and three cases with a normal spinal protein.

#### RESULTS OF PREVIOUS WORK

Work on trypanosomiasis was first begun in Liberia on March 24, 1941, and continued until May 20, 1943. All this work was done in the Western Province. The tribes in which trypanosomiasis was found were the Kissi, Gbandi, Loma, Mendi, Belle, Mandingo and Kpelle. Of these, the Kissi and Gbandi were the two tribes from which most of the cases came. There was enough work done in these two tribes to make an impression on the rate of infection of trypanosomiasis. During my first term of service there were 7,535 patients from all tribes who received 8 or more treatments of tryparsamide or of antrypol and tryparsamide.

When the work was resumed in 1943-1944 under the American Foundation for Tropical Medicine, Inc., an effort was made to find out the effect of the previous treatments given to these patients. A special form was printed to check the results. On this form spaces were provided for the following: 1. Name. 2. Card number. 3. Father's name. 4. Age. 5. Sex. 6. Drugs used. 7. Dosage. 8. Date of first injection given. 9. Number of months after treatment, when the check-up was made. 10. Condition of the patient, after treatment. This information gave a fair estimate of the patient's progress after treatment.

When the workers and the guards were sent to gather the Chief of the town and his people, they were instructed to have all the people who had had treatments previously, bring their cards. The response was satisfactory, one reason being that if a person could prove that he had received treatment, he would be excused from any further treatments in the future. The Chiefs of the towns in most instances had collected the cards from the families in which deaths had occurred from trypanosomiasis. In many instances the chief could give useful information regarding the movements of patients. When a patient said he had had treatment but had lost his card, this case was not counted. When a patient did not have as many as eight treatments recorded on his card, the case was not counted in this series of cases.

When a patient presented himself with his card a very careful examination was made to see that the card belonged to this particular patient. This could be checked by the name, the father's name, age and sex. The exchange of cards was a point which had to be watched all the time.

Each patient was examined for gross enlargements of the cervical lymph glands. Small hard glands, very firm on palpation, are encountered in patients for months after all clinical symptoms have disappeared. In these examinations a large soft gland usually means that the patient has had a relapse, or a reinfection. A small hard gland usually means that the patient has recovered and that the resolution of the gland is not yet complete.

Each patient was asked especially about headache. This is usually the first symptom that is noticed when relapse occurs. The patients can note that the headache accompanying trypanosomiasis is different from that observed in malaria or other diseases of that area. The headache is different in that it is present at all times of the day. The taking of food has no effect on this type of headache. It is a constant, dull type of pain. No definite seat can be pointed out where its greatest intensity is localized.

Nutrition was noted in general on this group of patients. Those who reported that they were well and could see well, had usually gained weight, and showed good nutrition. The cases which had relapsed were usually poorly nourished.

The effect of the drugs on vision was observed in every case. The cases were divided into two groups, one of which was composed of those who were totally blind and the other of those who suffered from dimness of vision, but had recovered sufficiently to carry on their work. The state of the patient's vision was noted on the record of each case.

The total number of patients examined who had been previously treated was 2,764. Since the total number of patients examined in the first group of 1941-1943, who had received eight or more treatments, was 7,535, this figure of 2,764 represents 35 per cent of the group treated. A check of 35 per cent of the cases of the original group may be taken as a fair sample.

This group of 2,764 patients, examined during 1944 to find the results of previous treatments, came from three tribes. There were 2,387 from the Kissi tribe. The reason this number is large is that the entire Kissi tribe was seen and the total population examined. It is also the tribe from which the greatest number of cases were found in the first term of service. Only 334 cases were examined from the Gbandi tribe. This tribe does not cooperate well on medical problems and many of these people destroyed their cards. There were only 43 of the Loma (Buzzi) tribe, the cases in this section being scattered and difficult to locate.

Dividing this group of cases according to sex, we find that there were 1,377 males and 1,387 females. In the consideration of the group of patients seen from 1941-1943, the sex ratio of males over females was 1.02 on the per capita basis. There was roughly an even division of cases in regard to sex. The check-up on females is a little more complete than on males. Men continually go away to find work in industry.

When the cases were divided according to age distribution, the following was found:

AGE	CASES
<i>years</i>	
0-9	286
10-19	457
20-29	736
30-39	837
40-49	353
50-59	83
60 and over	10

It will be observed that the 30 to 39 age group is the highest in this series of cases. The same distribution was found in the group of cases treated in 1941-1943, so that the group of patients seen for re-examination was of approximately the same age distribution as the original group of cases.

The cases were next divided into number of months after treatment in which the second examination was done. The following figures were found:

	<i>CASES</i>
From 6 to 11 months.....	11
From 12 to 17 months.....	159
From 18 to 23 months.....	366
From 24 to 29 months.....	1,198
From 30 to 35 months.....	795
From 36 to 40 months.....	235

There were 2,228 cases examined which had been treated two or more years previously. This period is probably long enough to consider the patient reasonably free from relapse. It probably means that the patient has recovered from the disease. However, in the French Colonies it has been the custom to keep all cases of trypanosomiasis under observation for a period of five years. This period of two years is probably not long enough to consider fully the results of treatment, but it does at least give some indication of the results.

In the consideration of the results of treatment, there were 2,609 cases which were well and had no visual disturbances at all, representing 94.35 per cent of the group examined. From the history and physical findings in these cases it was evident that these patients were well clinically.

The relapse rate of this group is high. In eighty-nine cases, or 3.19 per cent of this group, trypanosomes could be demonstrated. It is impossible to say whether these are new or relapsed cases. As I know of no way to separate these two conditions, I shall consider them all as relapsed cases. If the spinal fluid protein had been determined and a spinal fluid cell count done on these cases, some information might have been obtained. A high cell count with a low spinal protein indicates an early case. A relatively low cell count with a high spinal fluid protein indicates a far advanced and probably a relapsed case.

It is difficult to give an answer as to why these eighty-nine cases relapsed and why the other cases recovered. The records of these cases suggest no reason as to age group, sex, or tribe that might be a factor. Many of the relapsed cases were not considered far advanced during the first term of service. These patients may have had very high spinal fluid protein when the original course of treatment was started.

Since this group of eighty-nine cases improved clinically with a second course of treatment, it is possible that the principal reason why they relapsed was insufficient treatment. None of these cases were found to be arsenic fast, and none of them showed severe reaction to antypol and tryparsamide.

Since there were forty-three known deaths in this group of patients and since these deaths were the result of trypanosomiasis, it seems fair to include these deaths also in with the relapsed cases. The group of relapses would then include the eighty-nine cases in which trypanosomes were found, as well as the forty-three deaths, making a total of one hundred and thirty-two cases. Relapse occurred in 4.81 per cent of the cases treated. However, since trypanosomiasis is a rather chronic disease and often acute respiratory diseases or dysentery is the final cause of death, it is difficult to say in this group of patients whether all the deaths were due to the relapsed trypanosomiasis or intercurrent disease. A relapse rate of 4.81 is a rather high rate, but probably fairly correct.

The effect of tryparsamide on the optic nerve and the resulting dimness of vision or blindness has been one of the reasons much caution has been used in giving this drug. A distinction should be made between dimness of vision and total blindness. In the group of patients studied there was one case of total blindness. This represents 0.03 per cent of the cases. The cases that result in blindness are nearly always cases of patients well advanced in years, who have arteriosclerosis and are usually longstanding cases of trypanosomiasis. With most of these cases it becomes a choice of saving the patient's life or saving his vision.

There were twenty-two cases or 0.79 per cent of the cases which showed some dimness of vision. Two types of reactions are noticed in patients from the effect of tryparsamide on the optic nerve. The first type of reaction comes after the first or second treatment with the drug. The patient on the following morning is blind or nearly so. He may be able to notice moving objects or bright lights. The reaction is sudden in onset and severe in type. Drugs such as calcium thio-sulfate do not improve the vision in these cases. Sometimes the patient is permanently blind; at other times a slight improvement comes after several months.

The other type of reaction comes after the sixth to tenth treatment. The onset of dimness of vision is gradual. A narrowing of the field of vision is noticed and the patients have to be *asked* in order to find out about this symptom because of its mildness. Another two grams of tryparsamide does these people no great harm. This group is usually able to finish treatment without resulting ill effects.

This may be summarized by saying that in the first group the tryparsamide acts as a severe toxic agent on the optic nerve. In the second group of patients



the tryparsamide action is slow and begins to cause trouble on the optic nerve when the body is saturated with the drug.

As noted above, in this group of cases studied there were forty-three deaths. Most of these deaths were due to symptoms of trypanosomiasis, occurring two or three months after treatment. The cases were divided about equally as to sex and about equally distributed in all the age groups. Fifteen patients also had dysentery at the time of death, and five women died from delivery of a child or a miscarriage. It is difficult to say what part trypanosomiasis had in these deaths. Practically all patients had some symptom or signs of trypanosomiasis at the time of their death.

### SUMMARY

#### *Report on 2,764 Cases*

Well, and see well.....	2,609 or 91.35 per cent
Relapsed cases.....	89 or 3.19 per cent
Dimness of vision.....	22 or 0.79 per cent
Deaths.....	43 or 1.58 per cent
Total blindness.....	1 or 0.03 per cent

### RESULTS OF DIAGNOSIS AND TREATMENT IN THE GBANDI TRIBE

During my first term of service in the Western Province of Liberia from March, 1941, to May, 1943, intensive work was done on the Gbandi tribe. There were 3,425 cases of trypanosomiasis diagnosed in this tribe and the greater part of them finished a full course of treatment.

On my second tour of service one of the first steps taken was to evaluate the results of this previous treatment program. In January and February, 1944, 334 of these earlier treated cases were examined and the results of the treatment and dimness of vision were carefully noted. The next step in the procedure in the Gbandi tribe was to make a survey and determine what the rate of infection was at that time. This was done in four of the largest towns of the tribe during January and February, 1944. The rate of infection was determined as 1.42 per cent.

Following this survey a diagnosis and treatment was begun and all the population of three clans examined with the result that 67 Gbandi cases were found. These three clans were in a section of the Gbandi Tribe where there had previously been a very high infection rate. The three clans were Vassala, Wamoma and Tahamba. The purpose of the survey in September, 1944, was to see if satisfactory progress had been made in the reduction of trypanosomiasis. There were 6 towns used in this survey, located in the 3 clans mentioned. They were Babahun, Botembah, Massambolahun, Porowu, Popalahun and Bolahun, the largest towns in this area. Among 1,333 persons examined, 6 cases of trypanosomiasis were found, the infection rate being 0.45 per cent. A reduction of 1% had taken place since February, 1944.

Trypanosomiasis is no longer of public health importance in the Gbandi tribe. It is doubtful if this low level of infection will remain if control measures are not put into effect soon.

*Survey of Gbandi Country*

DATE	NAME OF TOWN	NO. OF HUTS	NO. EXAMINED	MALES	FE-MALES	BLOOD FILM	NEG-ATIVE	POS-ITIVE	GLAND PUNCT.	NEG-ATIVE	POS-ITIVE	TOTAL POSITIVE
1944												
8/29	Babahun	55	166	79	87	39	39	0	13	13	0	0
8/30	Botembah	49	210	93	108	42	41	1	19	17	2	3
9/7	Massambolahun	203	396	196	200	49	49	0	32	31	1	1
9/8	Porowu	84	208	90	118	22	22	0	10	10	0	0
9/9	Popalahun	66	211	92	119	32	32	0	19	18	1	1
9/10	Bolahun	87	151	80	71	26	26	0	19	18	1	1
Totals .....		544	1,333	630	703	210	209	1	112	107	5	6

## RESULTS OF DIAGNOSIS AND TREATMENT IN THE KISSI TRIBE

The survey of the Kissi tribe was done from August 31 until September 5, 1944. Previously, during May, June, July and August, there had been an examination of all the Kissi tribe and 493 patients had been found to have trypanosomiasis. This group of patients was now treated, the only cases not receiving treatment being those who had moved from the area. A previous survey was done in this tribe during February 1944. The same towns were used in both surveys because these were the largest towns located in the tribe. The towns were Poloma, Bolelu, Sardu Pascia, Foyakamara and Kondobengo. The patients in the Kissi tribe had been treated in three treatment centers located at Bolahun, Poloma and Bolelu.

A careful study had been made to find all the relapsed cases and to do laboratory work on this group of patients.

There had also been an examination of 2,384 patients in the Kissi tribe who had been treated during the first period of work for 1941 until 1943. There were 1,532 persons examined in this survey, 691 males and 841 females. A total of 318 laboratory examinations were done. There were 114 cases in which the material was aspirated from the cervical lymph glands for examination. There were 201 examinations made from thick blood film preparations. As a result of this work seven cases were found positive by the gland puncture method and three cases found positive on the blood film preparation. The rate of infection for trypanosomiasis is 0.65 per cent.

This work shows that a substantial reduction in the incidence of trypanosomiasis can be made by a campaign of treatment of the infected cases. The rate of infection was reduced approximately 2 per cent in less than a year. It is difficult to bring about a further reduction after the incidence has reached the level of one per cent. New persons entering this area are largely responsible for this fact. Since it has been shown by a survey of the Western Province that trypanosomiasis occurs over a large territory with a low rate of infection this condition will probably not change. However, at the level of 0.65 per cent trypanosomiasis is no longer of public health importance. Unless some measures are put into effect to maintain the infection rate at this level it will probably become higher.

*Survey of Kissi Country*

DATE	TOWN	NO. OF HUTS	NO. OF EXAMS.	MALES	FE-MALES	BLOOD FILM	NEG-ATIVE	POS-ITIVE	GLAND FUNCT.	NEG-ATIVE	POS-ITIVE	TOTAL POSITIVE
1944												
8/31	Poloma	68	324	137	187	24	22	2	21	19	2	4
9/1	Bolelu	43	291	121	170	41	44	0	18	17	1	1
9/2	Sardu Pascia	90	321	146	175	48	48	0	25	25	0	0
9/3	Foya Kamara	88	382	192	190	43	42	1	29	27	2	3
9/5	Kondabengo	60	214	95	119	45	45	0	21	19	2	2
Totals .....		319	1,532	691	841	201	201	3	114	107	7	10

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## APPENDIX

### SCHISTOSOMIASIS IN LIBERIA

The first cases of schistosomiasis in Liberia were reported by Dr. Richard P. Strong and Dr. George C. Shattuck (1930). Their cases were of the *Schistosoma haematobium* and *Schistosoma mansoni* type of infection and were found among the tribes in the Interior and among the inhabitants along the coast.

Dr. E. Maass, in an article written in 1927 "On the Pathology of the Liberian Hinterland", reported that 33 cases were found at the Mission Hospital at Bolahun and that *Schistosoma haematobium* was widely distributed in that area. Both sexes were equally represented among the infected cases. *Schistosoma mansoni* was not found at that time. Later, in 1930, E. Maass and H. Vogel in an article on "Schistosomiasis Mansoni in French Guinea and Liberia", reported 3 cases of the type *Schistosoma mansoni*, contracted in Liberia some 50 miles east of the town of Bolahun. They reported no cases of *Schistosoma mansoni* coming from the Bolahun area itself.

In 1933, Harley reported 248 cases of schistosomiasis in the Central Province of Liberia near the French border. Both *Schistosoma haematobium* and *Schistosoma mansoni* were found, but there was a preponderance of *Schistosoma mansoni*. He further stated that schistosomiasis comes nearest to being an occupational disease, as women spend a great deal of time at the edge of pools washing clothes, and also bathe more in the streams than do the men. He stated, too, that chronic dysentery was seldom due to *Endamoeba histolytica*, but frequently associated with *Schistosoma mansoni*. Evidently the disease is fairly common in the Interior of the Central Province in Liberia.

A group of 23 schistosomiasis patients were studied at Bolahun in 1944. Twenty of these cases were *Schistosoma haematobium* infections and three were *Schistosoma mansoni*. Cases of *Schistosoma mansoni* had never before been reported from this area. All the cases of *Schistosoma haematobium* presented symptoms of the genito-urinary disease. Two of the cases of *Schistosoma mansoni* presented symptoms of rectal disease and the other case had mixed symptoms of both rectal and urinary disease.

In the group where the ova of *S. haematobium* were found there were 15 males and 5 females. The cases were all in 3 age groups. The age grouping of these patients is not important, because the group is too small and because there was some selection in that all pupils of a school were examined. The disease affects all ages and both sexes and not only this group.

My attention was called to the existence of this disease by the fact that boys from 6 to 8 years of age came in for treatment complaining of gonorrhea. It seemed unlikely that gonorrhea would be present in boys of that age and on examination of the urine the ova of *S. haematobium* were found.

AGE	CASES
0-9	4
10-19	8
20-29	8

Cases were found in 5 tribes of natives and were distributed very widely over this whole area.

TRIBE	CASES
Gbandi.....	10
Kissi.....	5
Buzzi.....	1
Mandingo.....	1
Mendi.....	3

The fact that 10 of these cases were from the Gbandi tribe does not mean that the disease is more prevalent in Gbandi country. The number of cases is small and the location of the medical station is in the Gbandi tribe. A large number of the pupils of the Bolahun School are of this tribe.

There was a wide variation in the duration of the disease in these cases.

CASES	MONTHS
3	0-5
3	6-11
9	12-23
3	24-30
2	60

The patients gave a history of from 5 months to 5 years for the duration of the disease.

Schistosomiasis is of a chronic nature and in the early stages very few symptoms are present. The fact that all our cases are in children or young adults makes a long history of duration of the illness impossible.

The cases were divided into two groups, those who were pupils of the Bolahun Mission School and those who were not. There were 12 school boys in this group and 8 other patients.

To determine the rate of infection a survey was made of the Bolahun School in which 96 boys were examined on April 28, 1944, and 12 cases were found. The infection rate for this group was 12.4 per cent. All the cases in the school group were cases of *Schistosoma haematobium*. It is difficult to estimate what the rate

of infection in the community really is, as the sample of school boys is hardly representative of the population. The students of the school were made up from 3 tribes, Gbandi, Kissi, and Buzzi, and came from all parts of these tribes and part of this group must have contracted the disease at their homes.

All the cases of boys attending the Mission School at Bolahun were counted as Bolahun residents, of which there were 13. There were 2 cases found in Massamabolahun, a town  $1\frac{1}{2}$  miles west of Bolahun, and 2 in Tawalahun, one mile east of Bolahun. Two of the cases were from the Kissi section, one from the market town of Diama in the center of the Kissi tribe and the other from the southern part of the tribe in the town of Langbabah near the Sierra Leone border. The other case came from the town of Vonjama, the government center of the Buzzi tribe and at least 45 miles distant from Bolahun. Vonjama is a very important center and natives come from all over the area when the court is in session and remain there sometimes for several weeks.

The symptoms reported by this group of patients were very similar. Pain is usually the first symptom mentioned and was present in the suprapubic region. A deep-seated perineal pain was sometimes described by patients early in the disease and most frequently came on after urination. Some patients describe the pain as a scalding sensation. Frequency and urgency of urination are nearly always present. Malnutrition, anemia and debility are commonly seen in the cases where the disease has been present over three years. Stricture is complained of in quite a number of cases, but does not occur in early cases. In practically all males there was a complaint of a drop of blood appearing at the end of urination. At times when the patient has been subjected to heavy work over a long period of time, blood would sometimes be mixed uniformly in the urine, which then presented a pinkish color. Fever was noted in only 3 of the cases and the pulse was usually normal.

In 3 cases the ova of *Schistosoma mansoni* were found:

*Case 1.* June 29, 1944. Name J. H., age 35, male, town Foya Kamara, Kissi Section. Symptoms: pain in left side of chest and in lower part of back; attack of diarrhea with some blood in stool. Duration of illness, 3 years. Examination showed thickening of mucous membrane of rectum with some papillomata. On palpation of prostate the patient complained of severe pain. No cysts of *Endamoeba histolytica* were found in the stool. Diagnosis was made from seeing ova of *S. mansoni* in urine.

*Case 2.* Name Finela B., age 29, female, from Kissi Tribe. Symptoms: attack of diarrhea with inability to pass a formed stool during quiescent period of diarrhea; pain in lower abdomen, especially after a bowel movement. Examination showed several papillomata in the rectum. Diagnosis was made by finding ova of *S. mansoni* in the stool.

*Case 3.* R. M., Americo-Liberian, age 40, female. Symptoms: marked malnutrition, anemia and debility; puffiness of abdomen, with enlarged liver and spleen. Rectal examination disclosed a thickening of the mucous membrane with papillomata forming a sort of stricture in the rectum. The principal difficulty in differential diagnosis was to eliminate malignancy. Diagnosis was made by finding ova of *S. mansoni* in the stool. This case was probably one of mixed pathology of the rectal and urinary type.

All the cases of *Schistosoma mansoni* seen were far more severe than those of *Schistosoma haematobium*. Pain in the abdomen was the most common of all

complaints. Attacks of diarrhea occur at intervals and then subside. Where there is a marked thickening of the rectal wall and the formation of papillomata, constipation is a common symptom. Fever is more common, while anemia, debility and malnutrition always are present in advanced cases of *S. mansoni*. Swelling of the feet and puffiness of the face were noted in 2 of these cases. The general clinical picture of the infection of *Schistosoma mansoni* is by far the most grave.

Treatment was carried out by using tartar emetic. The first dose consisted of  $\frac{1}{2}$  grain of the drug in 5 cc. of freshly distilled water and the following 10 doses of 1 gr. each in 10 cc. of distilled water given twice weekly. No serious reaction occurred and all the cases showed improvement.

The intermediate host of *Schistosoma haematobium* is the snail *Physopsis africana* var. *globosa*, specimens of which were found in the Western Province of Liberia at the following places. The identifications were made by Dr. Joseph C. Bequaert.

1. Kolahun, Gbandi section, headquarters for the Assistant District Commissioner.
2. Bolahun, Gbandi section, where the Holy Cross Mission is located.
3. Babahun, Gbandi section, 4 miles northeast of Bolahun.
4. Bawolahun, large town on the Kahar (or Kaia) River, 10 miles south of Bolahun.
5. Between Massambolahun and Porowu, in the Gbandi section.
6. Poloma, in Kissi section. This town is in the eastern part of the Kissi tribe.
7. Near Foyakamara, in the Kissi section, in the market town of Dama.
8. Vonjama, Buzzi tribe, residence of the District Commissioner.

Dr. Bequaert also collected this snail near the village of Bandemi, about midway between Foyakamara and Sodu, Kissi section.

The snail *Lymnaea natalensis* var. *undussumae*, the intermediate host of the large cattle fluke, *Fasciolopsis gigantica*, was found at Kolahun, Liberia, in a pool from which the people get their drinking water. According to Dr. Bequaert, this snail is very rare in Liberia, although H. Vogel in 1932 reports finding it at a few places in the Bolahun area.

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## PART II

### TSETSE-FLIES IN LIBERIA: DISTRIBUTION AND ECOLOGY; POSSIBILITIES OF CONTROL

BY JOSEPH C. BEQUAERT

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The present account of the distribution and ecology of tsetse-flies (*Glossina*) in Liberia is based primarily on observations made from December, 1943, to August, 1944, for the American Foundation for Tropical Medicine, Inc. I have included some information obtained on an earlier brief stay in the same territory, from July to November, 1926, as a member of the Harvard African Expedition under the leadership of Dr. (now Colonel) Richard P. Strong.

It seems essential to introduce the discussion of the Liberian tsetses with a concise description of environmental conditions. Of the twenty-one species of *Glossina* generally recognized at present, only four are efficient vectors of human trypanosomiasis, while the others are negligible, not being positively known to transmit the disease. The occurrence, abundance and habits of each species are closely correlated with the outside factors, or the environment, particularly as they concern the spread of the disease. Of these factors, the type of vegetation is of particular importance, and this in turn depends chiefly upon climatic conditions and to a lesser extent upon the physiography and nature of the soil.

#### THE LIBERIAN ENVIRONMENT

1. *Physiography*.—The Republic of Liberia, with an estimated area of some 43,000 square miles, has an Atlantic coastline of nearly 350 miles, stretching in a southeasterly direction (approximately between 11°30' W.G., 7° N. and 7°30' W.G., 4°30' N.). In the hinterland, the northwestern corner reaches to about 8°30' N., some 150 miles from the coast.

The coastal area, to some 20 miles inland, is generally flat and cut by many tidal lagoons and creeks, in which the rivers empty, their mouths being usually obstructed by shifting sand bars. In Liberia, the coastal lagoons are generally small, the largest being that of Fisherman Lake at Cape Mount. On this low plain, a few hilly promontories stand out as conspicuous landmarks: Cape Mount, reputedly 1,000 to 1,100 ft. above sea-level; Cape Mesurado (Mamba



Point) at Monrovia; Baffu Point; and Cape Palmas. Beyond the narrow coastal strip, gently undulating hills of moderate height (on the average 300 to 900 ft. above sea-level) cover most of the Republic. In a few areas only do these hills reach greater heights and a more rugged appearance, so that they might perhaps be called mountains, as the local inhabitants do. In the northwestern corner (Kolahun and Zorzor Districts) some of the hills reach 1,400 to 2,200 ft. The most conspicuous range, to the south of the native town of Pandamai (or Kpandemai), is said to reach 4,528 ft. On some maps this is called the Walo Mts., on others the Wolagwissi Mts., and the summit itself the Wullibih.<sup>1</sup> Notwithstanding the altitude, Dr. Veatch and I found conditions much the same on this mountain as in the surrounding lowlands, the summit itself being covered with dense rain forest. However, some other high points in the Kolahun area, known locally as "Fossas," are bare of vegetation, owing to the rocky and flat table-like nature of the top. Two of the largest "Fossas" visited on my journey are the Mpaka Fossa, between Bolahun and Kolahun, about 1,960 ft. high;<sup>2</sup> and the Ballawallah, about 10 miles south of Bolahun, which reaches 2,300 ft. Another important mountain group, in the region of Sanokwelle, occupies part of the north-central corner, close to the border of French Guinea. It is sometimes called the Nimba Mountains and is reputed to attain altitudes of 6,000 ft. or more; but its highest points are north of the border in French territory. Mt. Bobei, in the Liberian part of this range, is about 3,400 ft. high. According to the most reliable information, the Liberian section of the Nimba Mountains is also covered to near the summit with rain forest. In connection with the orography of the country, the point of most interest is the absence in Liberia of a sizable area where climatic or vegetational conditions might exclude tsetse-flies. No section of the country can therefore be regarded as naturally immune, owing to the lack of vectors, either to human or to animal trypanosomiasis.

Numerous rivers and streams drain the abundant rains. None of the rivers, however, are very large, many being only a few hundred yards wide. Only the following reach a greater width, at least in the coastal plain, all of them running more or less parallel to one another in a general southwesterly direction. The Mano or Gbea (Bewa) River forms part of the boundary of Sierra Leone. The Loffa River ends at Little Cape Mount, a few miles east of Fisherman Lake. The St. Paul River, which empties just north of Monrovia, is some 200 miles long and connected at its mouth, by means of the shallow Stockton Creek, with the Mesurado Lagoon, on which Monrovia is situated. The St. John River and Cestos River both start their course in the mountain range of the Sanokwelle District. The Sanguin River, Sino River and Grand Cess River are much shorter than the foregoing. The Cavalla (or Cavally) River, the largest of all, forms nearly a third of the boundary between Liberia and the Ivory Coast. The smaller of the two Firestone Rubber Plantations is situated

<sup>1</sup> At Pandamai I was told that the Buzzi call the entire range "Wolagwissi" and the summit proper "Mwuntebe".

<sup>2</sup> The Mpaka Fossa is shown in a book by P. Germann: *Die Völkerstämme im Norden von Liberia*, (Leipzig, 1933), Pl. XIIa.

on the right bank of the Cavalla, a few miles inland from Cape Palmas. Harbel Plantation is close to Monrovia in the drainage of the Du (or Dukwa) and of the Farmington, two relatively small rivers ending in a common mouth at Marshall. Even the largest rivers are over most of their course shallow, swift-flowing and obstructed by rocks, rapids or falls, which render navigation impracticable even for dugout canoes, except in the lower reaches on the coastal plain. The water level, moreover, fluctuates considerably with the seasons. After a prolonged drought (usually in March–April), the waters recede, exposing wide stretches of shaded sandy or rocky shores, which afford ideal breeding places for several species of tsetse. When the waters rise after the heavy rains (from August to December), they flood the banks, making them unsuitable to the breeding of the flies. The importance of this seasonal factor in the ecology of Liberian *Glossina* will be discussed later.

Geologically speaking, Liberia occupies one of the oldest sections of Africa, the core of the land consisting everywhere of Archaean and mostly metamorphic rocks, particularly gneiss, gabbro, granite and quartz. Various iron ores and mica-schists are also common. In the more rugged sections these rocks are often freely exposed at the tops or on the steeper slopes of the hills. Elsewhere they form outcrops in the beds of the rivers. Over much of the territory, however, the older rocks have been weathered and leached into a layer of reddish loam, often with limonitic concretions. The loam is particularly thick where it is well protected against erosion by the natural forest cover. Otherwise it is washed away or deteriorates into a hard crust of lateritic rock, particularly in flat areas which become water-logged after the heavy rains. Several such plains of lateritic rocky soil, devoid of trees and mainly covered with coarse grass, occur near Pandamai at the western foot of the Wolagwissi Range. No tsetse are to be found in them, since the lack of shade makes them unfit to the adult flies. Unfortunately the poor soil precludes any possible use of these plains for native settlements away from the haunts of tsetse-flies. In the coastal plain, the level areas have a thick cover of alluvial sand and loam deposited by the river floods or in the tidal lagoons.

2. *Climate*.<sup>3</sup>—The climate is typically tropical over the entire territory of Liberia, being characterized by a uniformly high temperature, a heavy rainfall and a high atmospheric humidity.

At Harbel Plantation, close to sea-level, observations carried on from 1932 to 1939 show an average yearly mean air temperature in the shade of 78.2° F., with an average of 7.4° F. above and below this point, or a total range of 14.8° F. The maximum absolute temperature on record was 96° F. on March 16, 1938, and the minimum 58° F. on January 21, 1939. The seasonal fluctuations are very slight. For 1932–1939 the lowest average mean monthly maximum temperature was 80.5° F. in August and the highest 89.4° F. in February. The

<sup>3</sup> The meteorological data for Harbel Plantation were kindly supplied by the Research Department of the Firestone Plantations Company, through Dr. K. G. McIndoe. Those relating to the Bolahan region were supplied by the Holy Cross Mission, through Father Leo F. Kroll.

minimum temperature varied much less, however, with the average monthly mean highest in May (71.8° F.) and lowest in January (69.3° F.). The highest average solar temperature ranges from about 122° F. in August to about 148° F. in April, with a recorded highest maximum of 165.5° F. The earth temperature at a depth of 1 ft. below bare soil shows a yearly average of 80.4° F. (average maximum of 83.5° F. in March–April, and average minimum of 76.6° F. in August). At a depth of 4 ft. the yearly average rises to 81.5° F. (from 84.3° F. in April to 78.9° F. in September).

The temperature appears to be much the same throughout the coastal area. Travelling inland, however, it changes gradually with increasing altitude, although always retaining tropical characteristics. In the region of Bolahun (1,640 ft. above sea-level), fragmentary observations for the year 1923–1924 gave an average yearly air temperature in the shade of 72.5° F., with an average of 6.1° F. above and below this point, or a total range of 12.2° F. March had the highest average monthly maximum temperature (88° F.) and January–February the lowest average monthly minimum (56° F.). This region is therefore decidedly cooler throughout the year than the coastal belt and the seasonal fluctuation of the temperature is more pronounced.

The rainfall is very heavy, in fact heavier than in most other parts of Africa with the exception of certain areas in Cameroon.<sup>4</sup> In the coastal belt it rains throughout the year, but the rainfall is unevenly distributed, being about four times as large during the “wet” season (from May to October) as during the so-called “dry” season (from November to April). There is also much irregularity from year to year in the total rainfall as well as in its seasonal distribution. At Harbel Plantation the average total annual rainfall for the eleven-year period 1932–1942, was 138.04 inches (maximum 188.85 inches in 1933; minimum 115.02 inches in 1937). The bulk of the rain usually fell during the six months from May to October (for instance, in 1933, 154.59 inches, or 81 per cent of the total, as against 34.26 inches, or 19 per cent, during the six months January to April and November–December). As a rule the wet season had two peaks, one about mid-June and the other about mid-September, but this feature was irregular (in 1933, the wettest month was August with 37.58 inches, the next wettest June with 35.36 inches). January and February were the two driest months (with 2.11 and 2.30 inches respectively in 1933). The lowest monthly rainfall on record during 1932–1942 was in January, 1937, with 0.02 inch, the next lowest in February, 1938, with 0.1 inch. The beginning and end of the heavy rains are usually heralded by thunderstorms sometimes with gusts of wind. During the wettest months it often rains almost continuously for several days and nights, there being then very little sunshine. The heaviest rain recorded in 24 hours was 9.6 inches on July 20, 1935, and the largest amount in one hour was 3.5 inches on July 8, 1934. It should be noted that at the coast there is always much cloudiness, even during the “dry” season.

Even in the coastal plain the rainfall varies from place to place, differing

<sup>4</sup> At Bibundi, on the slopes of Mt. Cameroon, the total annual rainfall may reach 400 inches.

sometimes appreciably in localities only a few miles distant. Certain sections of Harbel Plantation, for instance, receive more rain than others or than Monrovia. Going inland, the "dry" season becomes progressively more pronounced, while the total annual rainfall decreases, but the monthly distribution follows much the same pattern as on the coast. At Bolahun, in the extreme northwestern section, a total of only 92.82 inches were recorded from April, 1923, to March, 1924, of which 69.01 inches, or 74.3 per cent, fell from June to November, and 23.83 inches, or 25.7 per cent, during April to May and from December to March. The lowest rainfall was in January, with 1.61 inches, the highest in July, with 16.4 inches. Sometimes the dry season is nearly rainless in this area. During my stay there in 1944, no rain fell for about a month (from January 20 to February 19). On February 20 there was a thunderstorm with a high wind, but very little rain. For the next four weeks there were similar light rainstorms of short duration, with thunder and wind. In this northwestern section there is also much more sunshine during the "dry" season than at the coast. However, a peculiar haze often clouds the sky at that time of year, when the Harmattan, a warm and dry northeast wind loaded with fine dust, blows from the Sudan.<sup>5</sup>

The more prolonged drought in the hinterland of the Republic, at the sources of the main rivers, is important in many respects. In the first place, it causes the great seasonal fluctuations in the level of the water courses mentioned before. It has also a decided effect upon the vegetation. In fact, a line drawn from Kolahun to Vonjama marks about the northern limit of the continuous rain forest belt, being itself in a transitional zone where the forest is cut up by patches of grassland (savanna). North of this line savanna encroaches more and more upon the forest, until on the south bank of the Makona River, at the French Guinea boundary, grass formations cover most of the country, with the forest trees restricted to narrow fringes along the rivers. Both these features have an important bearing upon the distribution of tsetse in this area.

Throughout most of Liberia, the prevailing winds are from the southwest. From May to October the southwest monsoon blows regularly, being responsible for the heavy rains. The sudden high winds, which mark the end and beginning of the heavy rains (October to December and March to May), usually blow from the northeast.

In the coastal area the relative atmospheric humidity is high at all times. Observations at Harbel Plantation show that in all months it reaches 98% to 100% at night (from 11 P.M. to 6 A.M.). It decreases from about 7 A.M., reaching a minimum between 11 A.M. and 3 P.M. There is less decrease during the "wet" season. The average humidity falls below 75% only from December to April, during the "dry" season. The lowest relative humidity on record was 51% on January 8, 1937, between 2 P.M. and 3 P.M., during a typical Harmattan. The very high atmospheric humidity accounts for the heavy mists which frequently blanket the landscape in the morning hours (from

<sup>5</sup> The effects of the Harmattan are sometimes felt slightly even at the coast of Liberia toward the end of December and throughout January.

6 to 8 or 9 A.M.), even in the "dry" season. No records of relative humidity are available for the hinterland, but it may be supposed that it is much lower in the Kolahun-Vonjama area than at the coast, particularly as the effects of the Harmattan are more pronounced there. The relative humidity of the air is the most important single climatic factor affecting the ecology of tsetse. In Liberia it offers nearly everywhere very favorable conditions for these flies.

3. *Vegetation*.—The climate, as described above, is ideal for so-called "tropical rain forest", which requires a minimum annual rainfall of 76 inches, distributed fairly evenly over the several months. No doubt, forest was the original vegetation of most of Liberia, but this has been greatly changed by human activities in many places. It would be misleading to start my account with a description of true or primary rain forest, since it is not the type with which the traveller becomes first acquainted. In fact, most Caucasian residents probably never see it at all, even after several years' residence.

If a visitor goes ashore by surf-boat, as was the general custom until a few years ago, he first meets with an assortment of straggling grasses and weeds on the upper stretches of the beach. These we may overlook as of no interest to us. Beyond the beach our visitor will soon strike some of the tidal lagoons and creeks, the muddy edges of which are densely covered with "mangrove", a peculiar woody brackish water vegetation of the tropics. Its most conspicuous member is the mangrove-tree (*Rhizophora mucronata*), remarkable for the maze of aerial roots which descend from the branches and, after anchoring in the oozy bottom, develop into props supporting the tree. The mangrove fringes the lower course of the rivers for some distance inland, until the salinity of the water becomes too low.<sup>6</sup> Its dense shade affords ample protection for adult *Glossina palpalis*, but no appropriate breeding places, as the banks are muddy and flooded at high tide. The tsetse found lurking in the mangrove thickets therefore all migrated to them after hatching on higher ground. The mangrove swamps of Liberia are rather limited in extent, whereas farther east on the West African coast they tend to spread over very large areas.

Vegetation of the rain forest type probably originally reached close to the sea-shore in Liberia. At present, however, little of it remains in the narrow coastal belt, some 5 to 10 miles wide, owing to continued cultivation for many centuries. In this belt the higher, uncultivated areas are mostly open grassland (savanna), occasionally of the "orchard-like" type, with scattered low trees or bushes (such as *Anona senegalensis*, *Parinarium macrophyllum*, *Lonchocarpus macrostachyus*) or with patches of bracken fern (*Pteridium aquilinum*). The grasses are rather low on sandy areas, where the alang (*Imperata cylindrica*) often rules, but on loam or lateritic soil they are much higher and belong to several species of *Andropogon* or to elephant grass (*Pennisetum guineense*). Lower and damper stretches bear dense thickets of a variety of small trees and

<sup>6</sup> Fisherman Lake, now the port of entry for most visitors, was originally fringed with mangrove forest, which has now been cleared away in the near vicinity of the Airport. Some of the photographs published in H. Johnston's well-known work, "Liberia", were taken in mangrove forest (vol. 1, figs. 40, 172, 176 and 178).

creepers or of false date-palm (*Phoenix reclinata*). From the air this type of vegetation is seen along the entire coast, from Fisherman Lake to Cape Palmas, but the width of the grassland belt varies. As might be expected, it is widest near the main Libero-American settlements, where cultivation has been most extensive and most constant. It should be noted that these grass formations are frequently burned over at the end of the "dry" season, thus preventing the return of true forest growth.

The typical vegetation of the coastal belt has many xerophytic characteristics, such as the prevalence of thorny bushes and cactus-like milkweeds (*Euphorbia*), being on the whole much drier than the several types of forest to be found farther inland. This ecological feature is of some importance in the epidemiology of trypanosomiasis in Liberia, where the disease is peculiar in being at present rare or almost absent in most of the coastal districts, particularly in the settlements of the Americo-Liberians. This seems to be due in a large measure to the type of vegetation prevailing in the coastal belt. None of the Liberian species of tsetse has become adapted to the relatively dry and unshaded conditions of the coastal savannas. The few flies occasionally seen near the coast occur only in isolated remnants of rain forest growth or in the fringing woods on the lower course of the rivers, whence they may migrate temporarily into the mangrove thickets of the tidal waters. Their numbers are often too small to present a serious public health problem.

The remainder of Liberia is mostly covered with forest, that is with vegetation in which tree-growth predominates almost to the exclusion of grasses. The so-called "farms", or areas actually under cultivation at any one time, are so reduced in extent and so scattered that they scarcely make an impression on the whole, for instance, when the country is viewed from the top of a hill or from the air. As elsewhere in tropical Africa, three main types of forest should be considered: primary rain forest, second-growth forest, and inundated forest. Special conditions prevail in each of these types, influencing the suitability as a habitat for the several species of tsetse.

In the Western Province, the only section of Liberia with which I am personally acquainted, the roads and main trails run at present mainly through second-growth woods at various stages of recovery. As this is the type usually seen by travellers, it will be described first. It is essential to realize that it is an artificial product of the steady inroads of native cultivation upon the original primary rain forest. Since native agriculture knows neither fertilizer nor crop rotation, the crop return declines steadily, eventually to near vanishing point. How soon this will occur depends in each case upon the original fertility of the soil. When the decrease in harvest becomes noticeable, the natives start a new "farm" (as cultivated land is called locally) either in untouched primary forest or, if none such is available, in old second-growth forest. The clearing of the land is done during the "dry" months and is very thorough, little being left of the original forest. Even if some of the larger trees escape the axe, they will mostly die within the next years owing to the changes wrought in the environment. When the field is abandoned later, it may be said therefore to be with-

out wild vegetation, except for the weeds that grew in the crops. At first these weeds take the lead, but they are soon eliminated by the arrival of woody settlers from the outside. These include bushes, small trees and creepers with quick means of dispersal, of rapid growth and of low soil and water requirements. Although most of the species may be found growing elsewhere under other conditions, they form in second-growth a distinctive association, the constituents being much the same throughout tropical Africa. The umbrella tree, *Musanga smithi*, scarcely ever is missing from this assemblage. Within 10 or 15 years the second-growth produces a dense but low forest, in which the trees rarely reach over 70 feet in height, except for an occasional survivor of the original primary rain forest. The trees are usually scattered and admit more light through the canopy than those of true rain forest. This allows a luxuriant, entangled undergrowth of bushes, herbs and creepers, more nearly like the layman's concept of a "jungle". The drier conditions and lack of deep shade of second-growth forest are inimical to most species of tsetses, and, of the Liberian species, only *Glossina fusca* seems to be able to find shelter and food there. Even *G. fusca*, however, probably does not find appropriate breeding places in this type of vegetation.

Botanists generally believe that second-growth forest in tropical Africa is replaced eventually by primary rain forest, if left undisturbed by man for 75 to 100 years. With the climate of West Africa primary rain forest is, on land above flood level, the "climax" formation, in which the plant growth achieves a natural equilibrium. The afforestation process is, however, extremely slow and it is doubtful whether it is ever completed in Liberia, where new "farms" are nowadays frequently started in the older patches of second-growth forest. In order to find untouched areas of primary rain forest it is usually necessary to leave the main trails. Comparatively little of it remains in the area between the St. Paul and St. John Rivers, except in some of the hills. Early in 1944 several patches of fine forest were traversed on the trail from Paiata to Salala, by way of Sanoyea, and specimens of *Glossina fusca* were captured in most of them. On the right bank of the St. Paul River, primary rain forest was also prevalent over many stretches of the trail from Dobli Island to Belleyella and Bolahun, the "farms" and second-growth woods being usually found close to the villages. The original rain forest seemed particularly well preserved in the hilly country near the upper Loffa River and between Pandamai and Zigida. Some of the valleys here form deep ravines, often with tree ferns or bamboo thickets. Both *Glossina fusca* and *G. pallicera* are common in forested stretches of this area. The Gola Forest, in the hinterland of Fisherman Lake, some 50 miles from the coast, is reputedly the largest rain forest area in the Western Province, but I cannot find that it was ever visited by a botanist, so that its characteristics are unknown.

In so far as I became acquainted with it, the Liberian primary rain forest does not differ essentially from that of other forested sections of tropical Africa. It is a mesophytic forest of evergreen hardwood trees, in appearance not unlike the mesophytic forests of the Middle Atlantic United States. The chief differ-

ences are the great variety of species of trees and bushes, many of them broad-leaved, the abundance of epiphytes (or air-plants) and the prevalence of creepers. Many tropical trees develop flattened buttresses which brace the base of the bole. In primary rain forest it is usually rather easy to walk through the undergrowth of herbaceous plants and low bushes, which is not often entangled or jungle-like, except at the edges of clearings. The tops of the trees form an uneven but close canopy, allowing little light and heat to reach the lower strata and the ground. The humid and relatively cool atmosphere as well as the diffuse light of the woody undergrowth provide seemingly ideal conditions for the adults of *Glossina fusca* and *G. pallicera*, two species of tsetses not particularly restricted to the banks of flowing water.

Few of the Liberian forest trees are very large, most of them not exceeding 50 to 100 feet in height. Some species, such as *Dialium Dinklagei*, *D. guineense*, *Brachystegia leonensis*, *Macrobium macrophyllum* and *Pterocarpus santalinoides* may reach 125 to 175 feet, or very exceptionally 200 feet. The bole of the trees is moderately thick, usually under three feet across, even in the tallest specimens. The Liberian primary forest is on the whole much less impressive than that of certain other parts of Africa I am acquainted with, particularly the eastern Congo Basin, where many trees reach 150 to 200 feet in height. The small size of most Liberian trees is no doubt due to the poor quality of the topsoil, which covers the rocky subsoil with a thin layer. Sometimes the rocky core is so close to the surface that the trees remain stunted and, for lack of soil moisture, they may even shed the leaves in the "dry" months, as I observed during January and February on some of the hilltops in the region of Bolahun. I have seen nothing, however, in Liberia that could be called a "monsoon forest", such as occurs in the hinterland of Nigeria, farther east.

Where the soil is periodically flooded after the heavy rains and remains under water for most of the "wet" season, the usual types of rain forest trees do not grow. They are replaced by an impoverished type of woody vegetation, consisting of a few species of rather low trees, but with a greater variety of creepers. Thickets of *Raphia*, or piassava palms, are particularly characteristic, as well as climbing palms, or rattans, often provided with whip-like leaf-ends bearing sharp hooks. The kapok or silk-cotton tree (*Ceiba pentandra*) is one of the few very large trees that thrive equally well in inundated forest and in primary forest. Swampy forests are common in Liberia, either in the many depressions between the hills or along the banks of the larger rivers. They are favorite feeding and resting haunts of adult *Glossina palpalis*, although the breeding places of this fly are never located there. It should be noted that there are no extensive tree-less permanent swamps, the papyrus or Egyptian bulrush in particular being unknown in Liberia.<sup>7</sup>

In the extreme northwestern corner, some 10 to 15 miles north of Kolahun, the forest belt ceases and is replaced by grassland over approximately 500 square miles. This "savanna" type of country extends, moreover, into the adjoining section of Sierra Leone and, on the north bank of the Makona River,

<sup>7</sup> In other parts of Africa the papyrus swamps are generally free of tsetses.



is characteristic of French Guinea. The grass is usually high, with certain tall species of *Panicum* and *Andropogon* (6 to 7 feet high) dominant and with occasional stretches of elephant grass (*Pennisetum guineense*). Bushes or trees are very few, but oil-palms (*Elaeis guineensis*) are fairly common, scattered in the landscape. In this section the banks of most rivers and medium-sized streams are rather steep and are fringed with a continuous dense growth of low trees and bushes. These fringing forests are rarely over a few hundred yards wide, yet offer a perfect shelter for *Glossina palpalis* and its breeding places. Where water stagnates in the "wet" season, there are more or less open swamps with a scattering of trees of a peculiar type (*Scarcocephalus*). In recent years the local natives have been planting swamp rice in many of these depressions, a practice which no doubt exposes them more to schistosomiasis (Compare Dr. Veatch's Appendix on this disease).

From a purely botanical point of view, Liberia lies within the Upper (or Northern) Guinea Forest District (or Sub-province), itself a section of the Guinean Forest Province. The families, genera, and even some of the species of plants are mainly the same as elsewhere in West Africa. The flora is, moreover, essentially that of the Central African forest area which extends across Cameroon and the Congo Basin to the shores of Lake Victoria.<sup>8</sup> Of the five species of Liberian tsetse, two (*G. palpalis* and *G. fusca*) have a similar wide distribution on the Continent; but the others (*G. medicorum*, *G. pallicera*, and *G. nigrofusca*) are nearly restricted to Upper Guinea.

4. *Animal Life and People*.—In a discussion of trypanosomiasis, animals are of interest as a source of food for the tsetse-flies and as a possible reservoir of the trypanosomes of human sleeping sickness. It should be emphasized that, no doubt owing to its exceedingly tenuous, needle-like proboscis, a tsetse never partakes of any food except vertebrate blood at any time during its entire life and that its sole source of water is also blood. So far as known, the fly could neither survive nor reproduce in the absence of a blood diet.<sup>9</sup> Feeding is restricted to the adult fly, the egg and larva developing inside the abdomen of the female until the maggot is full-grown and ready to transform into a puparium shortly after being voided. Only one larva develops at a time in the female fly, the rate of reproduction being therefore low as compared with that of most other insects where many eggs are laid at once. This handicap is compensated mainly by the long life of the adult and by the careful selection of the breeding sites where the larvae pupate, so that relatively few of the offspring fail to hatch.

Feeding is usually carried on in broad daylight, but a few species are also crepuscular or may even attempt to bite on moonlit nights. The flies attack their prey outdoors, usually in the woods or grasslands, sometimes in villages, on open porches, in sheds or in tents. Only exceptionally do they stray inside

<sup>8</sup> Additional information on the flora of Liberia will be found in R. P. Strong's "The African Republic of Liberia" (Vol. 1, Chapter XXXII, pp. 513-568, by David H. Linder). See also G. P. Cooper and S. J. Record, 1931.

<sup>9</sup> H. M. O. Lester and L. Lloyd (1928) have shown that the digestive enzymes of tsetse are adapted to the digestion of blood and not of plant juices.

huts or houses and they seldom attempt to bite there. In contrast to malaria, human trypanosomiasis is not contracted indoors. Tsetses seem to be particularly attracted by moving objects, which explains why they often enter automobiles and railroad carriages. This peculiarity accounts for the specimens of *G. palpalis* which are sometimes observed near dwellings at Harbel Plantation, the flies having boarded a car or a bus (particularly if it is loaded with natives) at some river bridge.

Although the diet requirements for successful reproduction vary with the several species, it may be said that for all tsetses the abundance or scarcity of adult flies in a given locality is regulated in the first place by the accessibility of an adequate supply of suitable vertebrate blood. In the case of *G. palpalis*, experiments in various parts of Africa have shown that the first larva is laid about three weeks after the female hatches; after this nine days elapse on the average between parturitions, under favorable conditions of temperature and atmospheric humidity. Newly hatched males must engorge before they mate and the females must do likewise before they can produce the first larva. After each larviposition at least one full meal of blood is needed before a new larva starts developing in the uterus. Upon hatching or after voiding a larva on a breeding ground, the female must therefore seek a host at once. As a female *G. palpalis* may live three months in captivity and possibly a few weeks longer in nature, she produces at most from eight to ten puparia throughout life, and many flies die no doubt long before they reach that number.<sup>10</sup>

The two sexes are bred in about equal numbers either from batches of "wild" puparia collected in nature or from "bred" puparia laid in the laboratory by captive flies. In nature, however, there is often a preponderance of one of the sexes, due to a variety of circumstances, such as a different mortality rate, the greater and more sustained aggressiveness of the males, the distance from the breeding grounds to which the females must travel, etc. Both sexes bite and suck blood and, being about equally susceptible to infection with trypanosomes (H. L. Duke, 1930), are both instrumental in the transmission of disease. Possibly even, males may be slightly more important in this respect, as they are never diverted from feeding by the other activities of the females, such as the search for breeding grounds and larviposition.

The mammalian fauna of Liberia is by no means deficient in species.<sup>11</sup> Most of the mammals are, however, small and usually intolerant of attack by blood-sucking flies. Many of them also have secretive or nocturnal habits, so that they are not often exposed to the bites of tsetses. Some of the monkeys and particularly the chimpanzee (generally called "baboon" locally) are attractive to tsetses when these animals descend to the ground or to the water's edge; but they probably catch some of the flies that attack them and, moreover, have

<sup>10</sup> G. D. H. Carpenter (1919), in Uganda, recaptured a female *G. palpalis* 182 days after it had been first taken, marked and released; but few marked flies are recaptured after 60 or 70 days.

<sup>11</sup> See the account of the mammals by G. M. Allen and H. J. Coolidge, Jr., in R. P. Strong (1930, vol. 2, Chapter XXXIII, pp. 569-622).

become so scarce in most districts that the flies do not often come upon them. The larger mammals, which originally provided the tsetses with part of their main diet, include the red river pig, the giant river pig, the pygmy hippopotamus, the water deer, the bushbuck ("red deer" of the Libero-Americans), the bongo (locally called "elk"), the dwarf forest buffalo (*Synccrus nanus*; known as "bush-cow"), the elephant, and several small antelopes mainly of the duiker group.<sup>12</sup> None of these occur nowadays in appreciable numbers, the few remaining individuals living mostly isolated or in pairs. The scarcity of game is no doubt due largely to persistent and unchecked native hunting over several centuries, particularly after the introduction of fire arms. Even small herds of elephant or buffalo are rarities, at any rate in the Western Province; and one is justified in disbelieving the reported greater abundance of these animals elsewhere in Liberia. At any rate, the Republic has nothing remotely comparable to the large herds of wild mammals found elsewhere in Africa and it is decidedly not "big game country". The general scarcity of mammals probably explains why comparatively few individual tsetses are observed at any one time, and perhaps even why so few species of *Glossina* are found in Liberia. The main diet of the flies seems to be no longer the blood of wild animals, but has shifted to human blood. This appears to be particularly true of *G. palpalis*, the Liberian vector of human trypanosomiasis. The possible reduction in the total number of flies, which may have resulted from the destruction of much of the game by man, was compensated by their having become adapted to a predominantly human diet, thus increasing the chances for the spread of sleeping sickness among the population. Having been led to this point of view by my field observations in Liberia, I was interested to find that E. Zumpt (1937) reached somewhat similar conclusions in the plantation district of Mount Cameroon.

Although Liberia has a sizable bird fauna, it is doubtful whether it contributes materially to the blood diet of the tsetses.<sup>13</sup> Most birds are small and, moreover, do not allow themselves to be bitten. In addition, many of them live in the canopy of the trees, normally out of reach of the tsetses, which do not travel as a rule more than ten to fifteen feet from the ground. Even the ground birds of the forest, such as francolins and guinea-fowls, and the waders and ducks along the river banks are probably bitten very rarely in nature, as they are too close-feathered.

Amphibians and smaller reptiles are probably never bitten by tsetses. It is well known, however, that some species of *Glossina* readily attack the larger reptiles, particularly those living in or near water. Crocodiles and tree monitors (*Varanus*) are known to be great favorites with *G. palpalis*, the blood of these reptiles being sometimes its main food, whenever available (W. F. Fiske, 1920, pp. 376-378 and 390-409). *G. palpalis* is also known to feed on various

<sup>12</sup> Two important hosts of *G. palpalis* in other parts of Africa, the true hippopotamus and the situtunga antelope, do not occur in Liberia.

<sup>13</sup> G. M. Allen lists 281 species and races of birds from Liberia in R. P. Strong's book (vol. 2, Chapter XXXIV, pp. 636-748).

snakes and on tortoises. It can evidently thrive and reproduce on reptilian blood alone, a factor to consider in any attempt at controlling this fly. However, there is no proof at present that any of the reptiles can function as reservoirs for human trypanosomes.

Some twenty years ago, the only domestic animals of Liberia were a few sheep or goats kept by the natives of the hinterland and some pigs occasionally raised in the Libero-American settlements, in addition to many small dogs. Sometimes a horse or a head of cattle was imported from French Guinea by some wandering Mandingo. More recently cattle appear to be on the increase in the Western Province, particularly among the Kissi and Gbandi, where one may find sometimes as many as a dozen animals in one village. It is not clear, however, what use is made of them nor how they have raised the economic standards of the community. In any case they are important as an additional supply of food for the tsetse, cattle being particularly attractive to *Glossina fusca*. At Degei, on January 14, 36 *G. fusca* were captured in the center of the village, away from any cover and about half a mile from water, while feeding on two cows. As the cattle are allowed to roam freely in the environs of the villages during the day, some of the tsetse they attract may follow the animals when they are herded back in the evening. If *G. palpalis* infected with *Trypanosoma gambiense* were thus carried into the towns, the keeping of cattle might contribute to the further spread of human trypanosomiasis, even though the animals themselves were not serving as reservoirs of the disease.

The rôle of wild and domestic vertebrates as natural reservoirs of *Trypanosoma gambiense* in Liberia has never been investigated. It is known, however, that some of these animals are important in this respect elsewhere in tropical Africa. Any serious attempt at prevention or control of the disease will therefore have to consider this problem in the future.

The foregoing considerations seem to point to man as one of the main, if not frequently the chief source of food for tsetse in Liberia under present conditions. In many sections, he offers a supply of blood more widely distributed, more abundant and more readily accessible than any wild or domestic vertebrate now living there. Both the density of the population and the customs of the people are important in this respect. These factors regulate the opportunities of the flies biting humans, hence of their becoming infected with trypanosomes and of their reinfecting healthy individuals. As no census has ever been made, not even of the Americo-Liberians, the total population can only be estimated by noting the distance between the villages or towns and the number of huts in each of them.<sup>14</sup> As stated by Dr. Veatch, in the Western Province (and in general in Liberia) the population is greatest in the coastal area and along the French border, while relatively few people live in the intervening districts. Dr.

<sup>14</sup> Sir Harry Johnston estimated the indigenous population at 2,000,000, no doubt an exaggerated figure. My own observations in the Western Province and Dr. Veatch's surveys suggest that about 800,000 to 900,000 people live in the entire Republic. From all accounts, the population is sparser in the Central and Eastern Provinces than in the Western Province.

Veatch estimates that the northwestern corner of the Republic is inhabited by about 85,000 persons of the Gbandi (23,000), Kissi (20,000) and Buzzi (42,000) tribes, in an area of some 3,800 square miles (approximately 23 persons per square mile). The country between the Loffa, St. Paul and St. John's Rivers, which I have traversed along the main trails on two occasions (1926 and 1944), is more sparsely settled. Here the villages are far apart and consist of only a dozen huts on the average. In 1944, moreover, the population seemed to have decreased since my first visit, 18 years earlier. Some villages were clearly decaying, to judge from the many abandoned huts; although, so far as I could discover, the natives had not moved away to new town-sites. Most probably the decrease in the population of this area is more apparent than real, being influenced by the recruiting of native labor for the Firestone Plantations Company.

It may be of interest, particularly for the possibilities of sleeping sickness control, to consider briefly various types of occupational exposure to the bite of, *Glossina palpalis*, the sole known vector of the disease in Liberia, present wherever indigenous cases have been discovered. It is the only species of *Glossina* known in the northwestern corner, beyond the forest belt (north of Kolahun), where the Kissi tribe shows at present a relatively high incidence of trypanosomiasis (rate of infection about 2.5 per cent, according to Dr. Veatch). Here this tsetse is restricted to the fringing forests along the rivers.

River travel is practically non-existent in Liberia, except on short stretches of some of the larger rivers in the coastal plain. This eliminates a source of infection which plays an important rôle in the epidemiology of the disease in other African territories, particularly in the Congo Basin.

In most parts of Africa, fishermen are more continuously exposed to tsetses than persons of any other occupation, hence often show a higher rate of infection. This factor again does not seem to have the same importance in Liberia. Professional fishermen are generally found on the coast only, where they engage in sea-fishing. Inland fishing appears to be carried on in desultory fashion, often only during the few weeks when the rivers are low. Under such conditions the hazard of contracting sleeping sickness from this source is probably not very great to the population as a whole. Hunting also is done by relatively few persons and, although occasionally river banks may be selected for the purpose, it more often will lead away from the water and from the haunts of *G. palpalis*.

In the hinterland of Liberia, the villages and towns are generally situated on high ground, often on a hilltop, surrounded by an extensive cleared area. They are therefore normally free of *G. palpalis*, except for stray specimens following people along the trails or brought in by roaming cattle. Many of the farms are established away from the water and, once the forest has been cut down, no cover is left for the tsetses. Normal native life in the towns and on the farms would seemingly offer little danger of infection, were it not for the close proximity to most towns of a river with densely wooded banks. Frequent visits are paid to this "water side", either for washing or bathing, or in order to obtain water for drinking and cooking. These spots and the many river crossings on

the trails, either by bridge, dugout canoe, or raft, are always frequented by tsetses. In all probability *G. palpalis* travels along the rivers towards such localities for the specific purpose of feeding on man.

Dr. Veatch rightly stresses that the stopping of the inter-tribal native wars and the development of roads and plantations produced new social and economic conditions which eventually led to the increase of trypanosomiasis. Until some 25 years ago, sedentary life prevailed among the tribes of the interior, few people ever leaving the confines of their clan or tribe. At that time trypanosomiasis appears to have been restricted to a few endemic cases, such as those diagnosed by Dr. M. Theiler in October, 1926, at the village of Betala.<sup>15</sup> Travel has become meanwhile a prevalent custom. It now includes, not only frequent movements of natives to and from market places (a common practice among the Kissi), but also portage over long distances of food supplies, particularly rice, from the interior to the coastal towns, and movements of recruited labor to and from the plantations.

By travelling over a wide territory, the relatively few cases of trypanosomiasis make it possible for an ever increasing number of *G. palpalis* to become infected at the river crossings. Although a low percentage of the flies biting a sick person ever acquire the infection, it should be remembered that an infected fly may live many months and that it remains infective throughout life. A single infective tsetse at a crossing may therefore inoculate several persons with the disease. If preventive measures against the spread of sleeping sickness in Liberia were to be carried out, fly control at the river crossings along the main trails would be the first and most important problem to deal with.

#### THE LIBERIAN TSETSE-FLIES

The tsetse-flies, or members of the genus *Glossina* Westwood, are highly specialized blood-sucking muscoid flies. Some 21 well-defined species are recognized at present, which have been classified into three groups here given subgeneric rank, following F. Zumpt's (1936a and 1936b) example: *Glossina* proper, *Nemorhina* Robineau-Desvoidy, and *Austenina* Townsend.<sup>16</sup>

In the course of my travels in Liberia in 1926 and 1943-44, I have observed only four species of *Glossina*;<sup>17</sup> but a fifth species has been recorded from a single locality on the coast. The more technical characters of these five tsetses are given below. Some of these characters are readily seen in the field with a hand lens; others need more careful study in the laboratory.

<sup>15</sup> This village, also called Betandu or Vezala, is situated a short distance from the left bank of the St. Paul River, about 5 miles south of Paiata. Trypanosomes were demonstrated in five cases of human trypanosomiasis in the hinterland of Liberia by the Harvard Expedition of 1926 (see "The African Republic of Liberia", vol. 1, pp. 497-498).

<sup>16</sup> These divisions are based on rather recondite characters. To raise them to generic rank, as C. H. T. Townsend (1937) proposed doing, would add little to our knowledge of the insects and merely confuse the medical man and the veterinarian. *Newsteadina* Townsend is here regarded as not separable from *Austenina*. The tsetse-flies are sometimes placed in a special family Glossinidae.

<sup>17</sup> It may be of some interest that all four species were taken in 1944 at Harbel Plantation, although they are very rare there.

- A. Smaller species, the wing 7 to 9.5 mm. long. Cerci of male terminalia (external parameres or superior claspers) connected by a thin membrane, which is deeply notched medially. External female terminalia of six plates; no signum internally. (Subgenus *Nemorhina*).
1. Third segment of antenna slender, strongly curved outward at tip, its anterior edge with a fringe of very long hairs (almost as long as the narrowest portion of the segment). Abdomen paler or darker brown, somewhat more yellowish medially and at the apical margins of the tergites. Antenna and most of hind tarsi yellowish or buff. *G. pallicera* Bigot.
  2. Third segment relatively short and broad, the tip very little projecting outward, its anterior edge with very short hairs, which are often difficult to see. Abdomen grayish-black, with very narrow pale apical margins of the tergites and with a poorly defined pale median stripe which is wider anteriorly. Antenna and hind tarsi blackish. *G. palpalis* (Robineau-Desvoidy).
- B. Larger species, the wing 11 to 13.5 mm. long. Cerci of male terminalia long, entirely free. External female terminalia of five plates; usually with a signum internally. (Subgenus *Austenina*).
3. Palpi relatively short, not longer than the greatest width of the head (in front view). Wings pale, with the thickened portion of the anterior transverse vein pale in both sexes. Harpes of male terminalia narrow, with a single pair of lanceolate appendages, not covered basally with a spinose membrane. Signum of uterus cordiform. *G. medicorum* Austen.<sup>18</sup>
  4. Palpi long, always exceeding the greatest width of the head (in front view). Third segment of antenna strongly curved outward at tip, its anterior edge with a fringe of very long hairs (nearly three-fourths the width of the segment). Harpes of male terminalia narrow, partly covered basally with a spinose membrane. Signum of uterus slightly developed. *G. nigrofusca* Newstead.
  5. Palpi long, always exceeding the greatest width of the head (in front view). Third segment of antenna moderately projecting outward at tip, its anterior edge with a very short fringe of hairs. Harpes of male terminalia broad and strongly serrated distally, not covered basally with a spinose membrane. Signum of uterus well marked, with a deep V-shaped constriction at one end. *G. fusca* (Walker).

There is as yet no evidence that any other species of *Glossina* occurs in Liberia. E. Roubaud (1922), on his map of the distribution of tsetse in West Africa, marks one locality for *G. longipalpis* Wiedemann at about the boundary of Sierra Leone and the northwestern corner of Liberia. This record was evidently taken from J. J. Simpson's map (1913), which bears the mark for *longipalpis* at Bariwalla near Bomaru, a locality in Sierra Leone a few miles West of Vahun.<sup>19</sup> *G. longipalpis* inhabits relatively dry areas covered with savanna forest. This type of grassland, with many trees scattered evenly in the grass and usually with an abundance of game, is rather characteristic for the Sudan and occurs nowhere within the present boundaries of Liberia. Nevertheless it may be useful to describe briefly this tsetse for the convenience of future workers in the field. *G. longipalpis* is a relatively small species (the wing being 8.5 to 9.5 mm. long) of the subgenus *Glossina*, proper. The abdomen is ochraceous-

<sup>18</sup> *G. medicorum* is here characterized after R. Newstead, A. M. Evans and W. H. Potts' (1921) description, as I have not seen specimens.

<sup>19</sup> I am inclined to doubt the correctness of J. J. Simpson's record for *G. longipalpis* at Bariwalla. He does not seem to have collected the specimen himself, as, in his narrative (1913, p. 175), he mentions for the region of Bomaru only *G. palpalis* and *G. fusca*.

yellow with dark brown, irregularly interrupted cross-bands. The third segment of the antenna is grayish, with the tip moderately recurved and with the anterior edge bearing a fringe of moderately long hairs (longer than in *G. palpalis* but shorter than in *G. pallicera*). The tarsi are buff colored with only the last two segments of all legs either entirely blackish or with blackish tips. In the male terminalia the cerci are much broadened (spoon-shaped), completely connected by a membrane and ending at one side in a large tooth. The external female terminalia are much reduced, with three rudimentary plates only.

The puparia of the tsetse flies are characterized by two conspicuous hemispherical lobes at the posterior end. Few other dipterous puparia have projecting posterior lobes and when present they are of a different shape. It is possible, to some extent, to identify the puparia of the different species of *Glossina*. Those of four Liberian species differ as follows:

- A. Large puparia, 7 to 9 mm. long. Space between the posterior lobes very wide, seen from above or below as wide as the lobes, the lobes enclosing a deep cup-shaped cavity.
  1. Space between the posterior lobes shallow, broadly V-shaped seen from above or below, the upper inner edge of the lobes not in the least angular.<sup>20</sup> *G. nigrofusca*.
  2. Space between the posterior lobes deep, shaped like a broad horseshoe seen from above or below, the upper inner edge of the lobes slightly angular. *G. fusca*.
- B. Small puparia, 5 to 7 mm. long. Space between the posterior lobes narrowly horseshoe-shaped seen from above or below, much narrower than the lobes.
  3. Posterior lobes rather low, in profile broader at the base than high. *G. palpalis*.
  4. Posterior lobes high, in profile about as high as wide at the base. *G. pallicera*.

The puparium of *G. medicorum* is as yet undescribed.

Previous to the work of the Harvard Expedition of 1926, the tsetse flies of Liberia were practically unknown, as may be seen from E. Roubaud's map (1920 and 1922), where the territory of the Republic is left nearly a blank, and from the few records mentioned by Newstead, Evans and Potts in 1924. In the following discussion I have listed all localities which have come to my notice to date (1945), including those based on my own collections or contributed by Dr. E. P. Veatch and Dr. G. W. Harley. Together they now give a fair picture of the distribution of the tsetse flies in the Western Province; but we are as yet almost wholly uninformed as to their occurrence in the Central and Eastern Provinces. It may nevertheless be surmised that the same species exist there, as environmental conditions are apparently similar throughout most of the Republic.

With respect to their ecological requirements, particularly with regard to vegetational cover, the several species of *Glossina* may be divided into three groups:

I. *Riverine or Riparian Species*.—These tsetse flies find their optimum conditions along the densely wooded shores of lakes or on the forested banks of the main rivers and their affluents, provided there be a fairly wide stretch of open water. They are not found along the smaller forest streams which are completely enclosed and shaded over by the trees. In districts covered with continuous rain

<sup>20</sup> The puparium of *G. nigrofusca* is very similar to that of *G. brevipalpis*, as figured by E. E. Austen (1911, p. 30, fig. 5A) and others.



forest, the flies do not travel far from the water course into the adjoining forest cover, normally not over 150 to 200 yards. On the other hand, they may be found even in relatively narrow fringing forests in districts mainly covered with savanna. The adult flies require dense shade and high atmospheric moisture and under natural conditions (in the absence of man) feed preferably on animals frequenting wooded river banks (crocodiles, monitors, wild pigs, pygmy hippopotamus, etc.). *G. palpalis* is the only Liberian tsetse of this group.

II. *Sylvatic Species*.—These tsetses also prefer densely forested country of the tropical rain forest type, but are not restricted to the wooded banks of open water. The dense shade provided by closely crowded trees is essential to them, though they do not require at all times as much moisture in the air as the riverine species. They may therefore occur even in forested hills, far from water, and are often seen sunning themselves along the trails. Their source of blood is also more diversified. Three Liberian species, *G. pallicera*, *G. fusca*, and *G. nigrofusca*, belong here. Of these, *G. fusca* seems to require less forest cover than the others, as it is sometimes found feeding in villages over half a mile from any woody growth.

III. *Savanna Species*.—These tsetses prefer grassland, usually with scattered trees, bushes or thickets, which provide a certain amount of light shade. They thrive even where the environment is very dry part of the year and are often associated with large herds of game which they attack even in the brightest sunlight. *G. morsitans*, *G. longipalpis*, *G. longipennis* (a species found in very dry thorn-bush country in East Africa), and *G. pallidipes* are typical examples. No species of this group is definitely known in Liberia; but *G. medicorum*, which I have not observed myself, is stated by Ingram to occur in savanna forest in Northern Ashanti (R. Newstead, A. M. Evans and W. H. Potts, 1924, p. 63).

#### *Glossina palpalis* (Robineau-Desvoidy)

As *G. palpalis* is the only proved carrier of human trypanosomiasis occurring in Liberia, this species will be discussed at greater length than the others. It is one of the most widely distributed tsetses, extending throughout Tropical Africa from the mouth of the River Senegal to Benguella, inland to about 14° N. in Upper Guinea, but to only 7° to 8° N. in the eastern Sudan. The eastern limits are roughly the Uganda-Kenya boundary and the eastern shores of Lake Tanganyika. In the south it stops short of the Katanga highlands and reaches about 9° to 10° S. across Angola. The area it occupies covers therefore fairly well the Guinean Subregion of the botanists, which is characterized by luxuriant evergreen tropical tree-growth, either as a continuous forest belt or as fringing forests along the rivers. Moreover, the *G. palpalis* area coincides at present almost exactly with the distribution of the type of human trypanosomiasis caused by *Trypanosoma gambiense*.

In the British and French colonies adjoining Liberia the distribution and habits of this tsetse are well known. In Sierra Leone they were investigated mainly by J. J. Simpson (1913), W. Yorke and D. B. Blacklock (1915*a* and 1915*b*), D. B. Blacklock (1923), J. G. H. Frew (1939), and R. M. Gordon and T. H.

Davey (1930). The species was found to be ubiquitous, but always closely associated with dense forest growth along the river courses or (at the coast) with mangrove forest. J. G. H. Frew states that it usually does not follow more than 150 yards away from water. He also notes that its density is small, except on some of the mangrove creeks of the southern coastal districts, where he believes the crocodile is the preferred host. On a brief trip to the Kissi country of northeastern Sierra Leone, as the guests of Dr. R. D. Harding (February 8 to 11), Dr. Veatch and I found *G. palpalis* on the Keya River (a small sub-affluent of the Sulimah River) near the village of Balahun, as well as at the ferry of the Makona River a few miles north of Kailahun. On the Keya the flies were fairly common and aggressive where a trail crossed the river by means of a hanging bridge of lianas. Near this spot a number of puparia were also obtained from densely shaded, dry, loose sand, covered loosely with dry plant litter.

In French Guinea and the Ivory Coast, *G. palpalis* was studied intensively by G. Bouet and E. Roubaud (1917) and E. Roubaud (1920, 1922). In the Ivory Coast, which is largely covered with dense tropical rain forest, the species is generally distributed and its habits are the same as in Liberia. In French Guinea, however, savanna predominates and *G. palpalis* is restricted here to the fringing forests, while its occurrence and density vary greatly with the seasons. Somewhat similar conditions prevail in a small portion of northwestern Liberia, south of the Makona River.

The specific characters remain remarkably fixed throughout the vast African area occupied by *G. palpalis*. Liberian specimens seem to average smaller than those taken in the Belgian Congo. Otherwise flies from Liberia look like others taken farther east. Occasionally color differences may be noted in certain districts; but, being not clearly correlated with the distribution, they offer no tangible means of dividing the species into geographical races. R. Newstead (1910) first separated specimens from Uganda from the usual West African form, on the basis of some slight, though presumably constant differences in the external male appendages (terminalia). Later Zumpt (1935a) defined another form from the shores of Lake Tanganyika on similar peculiarities. In his most recent work F. Zumpt (1936a, 1936b, 1940) recognizes three subspecies: (1) *G. palpalis palpalis* (or typical *G. palpalis*), of Upper Guinea and the coastal districts of Lower Guinea. (2) *G. palpalis fuscipes* Newstead, of the hinterland of French Equatorial Africa, the Belgian Congo and Uganda. (3) *G. palpalis martinii* Zumpt, of the shores of Lake Tanganyika and the southeastern Belgian Congo (mainly east of the Lualaba River). The three races have the same habits and, so far as known, show no difference with regard to their efficiency as vectors of trypanosomiasis. Their distinction is consequently of no practical importance at present. Nevertheless it may be stated that, on the basis of male terminalia, only the typical form of the species, *G. palpalis palpalis*, occurs in Liberia.

E. E. Austen (1911, p. 26) called attention to the fact that the two apical scutellar bristles of *G. palpalis* are very short in females from the Belgian Congo

and Uganda, whereas they are much longer in females from Gambia, Sierra Leone and Liberia. The males, however, have long apical scutellar bristles throughout the entire range of the species. I can confirm this observation after examining several hundred Congolese and Liberian flies. In females from the Congo the bristles are not only short, but also very thick, more like spines; in those from Liberia the bristles are always thin and hair-like, but they vary much in length, being sometimes as long as in the males.

*Previous Liberian Records.*—*G. palpalis* was first recorded from Liberia by E. E. Austen (1911, p. 26), but without definite locality. E. Roubaud (1922, p. 725, footnote) mentions it from the vicinity of Monrovia, according to Bouet, and also shows it on the map as occurring in the extreme northwestern corner of the Republic. J. Bequaert (in R. P. Strong, 1930, vol. 2, p. 991) caught it at Kolobanu, Lenga Town, Moala, Banga, Paiata, Bakrata, Memeh Town, Reppo's Town (=Reputa), Kakata, and the Du River; he stated that Bouet observed it on the wooded seashore at Cape Mesurado close to Monrovia. F. Zumpt (1935a, p. 146; 1935b, p. 333; 1936a, p. 558) lists the typical form at Cape Mount and in the Hinterland of Liberia.

*Observations During the 1943-44 Survey.*—At Harbel Plantation, *G. palpalis* is now very rare, being only seen in small numbers at a few spots along some of the rivers, particularly the Ba River. In the course of some six months' stay, only four specimens were obtained with the help of native boys. On March 29, one fly was captured inside the building serving as a hospital for the Sleeping Sickness patients, close to Duside Hospital. This building is situated on a hill far from any wooded river course. On August 9, another fly was taken biting a native in the open yard of the house where I was staying, about a mile from Duside Hospital. Again there was no water nor forest cover nearby. Most probably in these two instances the flies had travelled in some vehicle after entering it at a bridge. I never saw *G. palpalis* during my many wanderings through the rubber plantation. This type of tree-growth, consistently kept free of undergrowth, provides no adequate cover for this species of tsetse. The scarcity of *G. palpalis* on the Du-Farmington plantations in 1944 contrasted sharply with their relative abundance in the same locality in 1926, when the plantations were begun. It demonstrates the effectiveness in tsetse control of organized tropical agriculture on a large scale. Considering the low normal rate of infectivity of *G. palpalis* for *Trypanosoma gambiense*, the few flies of this species present nowadays at Harbel Plantation scarcely offer any danger to the native laborers and the white personnel. It is nevertheless recommended to isolate in well-screened wards all native sleeping sickness patients as soon as they are recognized. According to information received from Captain M. S. Briscoe, no *G. palpalis* are ever seen on Roberts Field, where the right bank of the Farmington River is thoroughly cleared; but a few are occasionally taken on the opposite left bank, which is as yet densely fringed with trees.

From Harbel Plantation to Bolahun, via Dobli Island and Belleyella (January 8 to 23, 1944).—*G. palpalis* was observed at nearly every fair-sized river on this route, but never in large numbers. It was usually necessary to linger for

15 to 30 minutes at a crossing in order to see half a dozen flies. Specimens were caught at Dobli Island (at the two crossings of the St. Paul River, seen in the dugout canoes used as ferries; one bit me on the ankle while waiting on the bank, before I noticed it), on the Garlo River (in the dugout canoe at the crossing), in the village of Degei (one biting man at 7 A.M.), at Kumbaeta, at Belleyella (on the small river used for washing and bathing by the towns people and the Liberian Frontier Force), at Guawarmarma, at Bellepalamu, at Jenne (at the crossing of the Loffa River), and at Bauwalahun (at the crossing of the Kaia River, also spelled Kahar or Kihar and called the Seribu in the lower part of its course).

Kolahun District (mostly Kissi country; January 23 to February 22).—In the course of our survey, Dr. Veatch and I observed adult *G. palpalis* at Bolahun (crossing of the Waow or Wah River, between the Holy Cross Mission and Massambolahun), between Sodu and Sardu Pascia (on the Meio or Maio River, an affluent of the Makona, near the village of Kiesana), at Foyabudu (on the Senja River, an affluent of the Makona), and at the ferry crossing into French Guinea on the Makona River (near Ma). At each of these spots surprisingly few adult flies were seen, although puparia were readily found at all of them. The low incidence of tsetse appears to have been seasonal, our survey being conducted during the driest weeks (no rain at all fell during that period). After my departure from this area, conditions seem to have changed with the onset of the rains. Dr. Veatch's trained native assistant obtained many more flies on the Kaia and Waow Rivers during September, October and November, when the rains were very heavy; at the same time puparia could no longer be obtained. As noted before, the northern portion of the Kissi country is mostly high-grass savanna. *G. palpalis* is only present here at the rivers, the wooded banks of which have nowhere been cleared, not even at the ferry leading into French Guinea, which is frequented daily by travelling natives.

Southward from Bolahun to Kailahun (February 15 to 19).<sup>21</sup>—The route followed the general course of the Kaia River, which was crossed near Bauwalahun and at Bondualahun. Adult *G. palpalis* were observed at both points. One was taken biting a native on the porch of a house in the village of Bondualahun, only a few yards from the uncleared banks of the Kaia. Specimens were also seen on the Wahwah River at Kailahun. During his earlier survey (1941–1943) Dr. Veatch obtained *G. palpalis* at Vahun, a frontier station close to Sierra Leone, west of Kailahun.

Bolahun to Zorzor, via Vonjama and Pandamai (Gbandi and Buzzi country; February 22 to March 12).—This tour was made jointly with Dr. Veatch. Our route was at first northeastward to the ferry into French Guinea, on the Makona River near Nyandamolahun. This was found heavily infected with *G. palpalis* and some were also taken on a small affluent near the town, the Gnilia (or Ilia) River. No flies were seen east from there to Vonjama, the trail being mostly

<sup>21</sup> The Liberian village of Kailahun should not be confused with Kailahun in Sierra Leone, the headquarters of the British District Commissioner for the northeastern section of that Colony.

through second-growth forest and part of the way a broad and graded road. From Vonjama we proceeded southward to Pandamai, then eastward to Zigida, and again southward to Zorzor. Some of this area is heavily wooded and very suitable to *G. palpalis*, which was observed at every important river crossing: at Daugomai on the Loffa River; at Nikabuzu on the Lawah River; and at Zigida on the Wejah River. In addition some flies, which evidently had followed the natives from the river banks, were captured in the villages of Lormai, Daugomai and Bokesa.

Zorzor to Harbel Plantation (March 13 to 20).—After leaving Dr. Veatch at Zorzor, I returned to the coast by the shortest route. Travel was too rapid for detailed entomological studies. *G. palpalis* was observed at Bosaghi (crossing of the Yeh River), at Taninewa (crossing at the Seleyeh River), at Paiata (crossing of the St. Paul River), at Betalu (or Betandu, where a specimen was caught in the village; this is of special interest, as some of the first Liberian cases of trypanosomiasis were detected here in 1926), and at Sanoyea (Sanoghie of some maps).

Ganta country.—Information about this area was obtained from Dr. G. W. Harley. Great numbers of *G. palpalis* were brought to him by the natives and the species appears to be common and sometimes even abundant on most of the rivers. This section is entirely within the rain forest zone; but, as it is densely populated, large stretches are now second-growth woods.

To sum up: in the hinterland of the Western Province, from the Boundary to some 40 miles from the Coast, all fair-sized rivers have their densely wooded banks infested with *G. palpalis*. The flies are a potential menace as vectors of human trypanosomiasis on the trails at all river crossings, no attempt at protective clearing have ever been made at any of them. The problem is still further complicated by that of the breeding places, which will be discussed later in this report.

#### *Glossina pallicera* Bigot

*G. pallicera* occurs in the West African forest of Upper and Lower Guinea, from Sierra Leone to the French Congo, but in the southern part of its range it is found only in the coastal area.<sup>22</sup> It is strictly a tsetse of the continuous belt of rain forest (or "high bush") and is absent from the fringing forests of the rivers in savanna country. This explains why it is fairly common in the Ivory Coast and Liberia, but not known from French Guinea and extremely rare in Sierra Leone. J. J. Simpson (1913, p. 187) states that he saw it only from a single locality in Sierra Leone, which he does not name nor mark on his map; while E. E. Austen (1911, p. 137) and Newstead, Evans and Potts (1924, p. 162) mention the locality Firo (near the right bank of the lower Mano River, a few

<sup>22</sup> *G. pallicera* has been reported from the Belgian Congo, but I am inclined to doubt its occurrence there. R. Newstead, A. M. Evans and W. H. Potts (1924, p. 143) state that the 3 specimens they saw from that colony had a short fringe of hairs on the third antennal segment, whereas in typical *pallicera* this fringe is very long. They nevertheless referred these Congo flies to *pallicera*, because of the identity of the genital terminalia.

miles from Liberian territory). J. G. H. Frew (1929) obtained a single specimen in southwestern Koinadugu. On the other hand, the species is common in the southern forested part of the Ivory Coast, as shown on E. Roubaud's map (1920, 1922) and by R. Newstead, A. M. Evans and W. H. Potts' records (1924). The species was first described from Assinie on the Ivory Coast.

*Previous Liberian Records.*—E. E. Austen (1911, p. 37) listed specimens collected in 1909 by A. Pearse at Tappoima, Suji and Bonnatown, all in the Western Province, near the left bank of the lower Mano River. E. Roubaud (1922, p. 725, footnote) stated that Dr. Bouet found *G. pallicera* near Monrovia. R. Newstead, A. M. Evans and W. H. Potts (1924, p. 162) repeated the locality Tappoima. J. Bequaert (1930, in R. P. Strong, *et al.*, vol. 2, p. 992) collected it at Betala, Reppo's Town (=Reputa), Banga, Kakata, and Bomboma (near Moala); he also stated that Dr. Bouet took it at the Mt. Barclay Plantation.

*Observations During the 1943-1944 Survey.*—At Harbel Plantation a male of *G. pallicera* was captured on July 16 biting a native at 9 A.M., near the entrance to Duside Hospital. This was the only specimen of the species seen in this locality during several months' stay. In most of the hinterland of Liberia, *G. pallicera* is widespread and moderately common, as shown by the following records. On the trail from Dobli Island to Bolahun: Zowolata, in primary rain forest, January 12, several biting the hammock carriers, from noon to 3 P.M.; Konikanga near Degei, biting people about 9 A.M., January 13; near Moylakwelle, January 15, about 9 A.M., biting me on the chin without my being aware of it; Guawarmarma; Jenne, where a large proportion of some 700 puparia obtained on the banks of the Loffa hatched *G. pallicera*; Gondalahun. In the region of Bolahun this species is very rare, only a few being bred from some 5000 puparia collected for us on the Kaia River. During the survey of the Kissi country, north of Bolahun, none were seen. The species was again taken on March 14 between Salayea and Bosaghi (south of Zorzor). In 1941-1943, Dr. Veatch caught some specimens toward the northern edge of the Gola Forest, at Vahun and Gelahun.

My observations show that in Liberia *G. pallicera* is always associated with primary rain forest (or "high bush"), over which it seems to travel considerably. It is usually found far from water along the trails, where it attacks people very readily, in my experience always during the daytime. Here also it may be observed playing and resting on the ground or on low vegetation in full sunshine. I have never taken it at river crossings or on the banks of water courses, although the females evidently migrate to the rivers to larviposit, as shown in the sequel.

#### *Glossina fusca* (Walker)

*G. fusca* is one of the few larger species of tsetse flies widely distributed throughout the tropical African forest belt. It is, however, primarily a fly of continuous rain forest and is only found in the fringing woods of the savanna rivers when these are fairly extensive. It occurs from Casamance across Sierra Leone, Liberia, Ivory Coast, Gold Coast, Dahomey, Nigeria, Cameroon, French

Congo and Belgian Congo, to the Cuanza River in Angola. Eastward it reaches the extreme western edge of Uganda in the Semliki Forest and on Lake Edward.<sup>23</sup>

J. J. Simpson (1912, p. 188) writes that *G. fusca* "favors dense vegetation and a moderately moist climate, and is to be found in Sierra Leone in the regions of densest forest growth; in fact, as the map will indicate, the delimitation of the forests is at the same time a delimitation of the areas where *G. fusca* occurs." He records many localities, mostly in the southern half of the Colony. J. G. H. Frew (1929) made similar observations. He mentions taking it in a village at night, when there was no considerable area of forest within at least a mile, so that its range of flight must be fairly large. He also states that it bites man more readily than *G. palpalis*, but is more easily caught. The Sierra Leone natives associate its occurrence with that of elephant, bush-cow and domestic cattle. R. Newstead, A. M. Evans and W. H. Potts (1924, p. 90) state that it is widely distributed in Sierra Leone. It is unknown from the hinterland of French Guinea, north of Liberia. In the Ivory Coast it is, however, a common species, as shown on E. Roubaud's map (1920, 1922) and by R. Newstead, A. M. Evans and W. H. Potts' (1924, p. 90) records.

*Previous Liberian Records.*—E. E. Austen (1911, p. 72) reported specimens taken by A. Pearse in 1909 at Boje, Dombolo (male taken on an elephant), Da, on road between Heye and Gondo, near Bukei, and at Simbek (one male caught buzzing around a candle at 8.30 P.M.); all localities in the Western Province near the Sierra Leone border. According to E. Roubaud (1922, p. 725, footnote), Dr. Bouet caught it in the vicinity of Monrovia.<sup>24</sup> J. Bequaert (1930, in R. P. Strong, *et al.*, vol. 2, p. 993) observed it at Banga, Kolobanu, Reppo's Town (=Reputa), Suah Koko, Bakrata, and Paiata. F. Zumpt (1936b, p. 332) saw it from the hinterland of Liberia.

*Observations during the 1943-1944 Survey.*—*G. fusca* seems to have survived in the few remaining patches of "high bush" in and near Harbel Plantation. On August 8 a female was taken biting a native on the leg at 6 P.M., in a workers' camp close to Duside Hospital. Captain M. S. Briscoe also caught a specimen in December, 1943, in a forest patch close to Roberts Field. In the Liberian hinterland it is evidently a common fly, as shown by the following recorded captures.

From Harbel Plantation to Bolahun.—*G. fusca* was observed almost daily: biting one of the hammock carriers near the village of Ngiblai, about 2 P.M., January 9 (between Lakrata and Dobli Island); ferry of Dobli Island on the St. Paul River; several biting man on the trail through primary forest on the right bank of the St. Paul, from 9 A.M. to 3 P.M., particularly near Vanyata and Zowolata; common, biting cattle in the village of Degei, throughout the day,

<sup>23</sup> The supposed occurrence of *G. fusca* in Kenya and Tanganyika Territory was based on misidentifications (see Swynnerton, 1936, p. 173).

<sup>24</sup> The marks for *G. fusca*, apparently placed within Northwestern Liberia on Roubaud's map, are due to an erroneous tracing of the boundary in that section. They were evidently based on J. J. Simpson's Sierra Leone records.

January 14; near Kumbacta, in primary forest; near Bellelyella, where puparia were obtained (see below); Fasima; Guawarmarma; and Bellepalamu.

Region of Bolahun.—A few specimens were taken by Dr. Veatch in the vicinity of Bolahun (mostly on the Kaia River), but the species is rare in this area. It was not observed at all by Dr. Veatch and myself farther north during our survey tour of the Kissi country. In 1941–1943, Dr. Veatch caught some *G. fusca* at Vahun and Gelahun, to the southwest of Bolahun, near the northern edge of the Gola Forest.

Bolahun to Zorzor and southward to Harbel Plantation.—*G. fusca* appeared as soon as extensive wooded areas were entered: Vasala; Lormai; Pandamai; between Salayea and Bosaghi; and near Sanoyea.

In Liberia *G. fusca* has much the same habits as in other parts of Africa. My observations show that, while it is a decidedly sylvatic species, it may travel and feed for some time in the open, away from woody cover, for instance in native villages. It is by no means rare in lightly shaded second-growth forest, although it probably does not breed there. It readily attacks man and appears to be more aggressive than *G. palpalis*, but this may be due not so much to its being more attracted by man, but rather to its larger size making it more conspicuous. It is certainly a bolder fly, which will fly directly to the neck or arms, whereas *G. palpalis* prefers to move cautiously toward the ankles or legs. Several observers have emphasized its nocturnal or crepuscular habits, but my own observations do not bear this out for Liberia. All my captures were made in broad daylight, some even at noon, in every case while attempting to bite.

### *Glossina nigrofusca* Newstead

*G. nigrofusca* is restricted to a rather small area in Upper (or Northern) Guinea, from Sierra Leone to British Cameroon.<sup>25</sup> In Sierra Leone it is extremely rare, being known only from one locality, Baima (or Baiiama, near Pendembu, the terminus of the Sierra Leone Government Railway, some 15 miles west of the Liberian border), as shown on J. J. Simpson's (1913) map and recorded by R. Newstead, A. M. Evans and W. H. Potts (1924, p. 97). It is unknown from French Guinea and occurs in the Ivory Coast only in a few localities south of 6° N., according to E. Roubaud's map (1920, 1922) and R. Newstead, A. M. Evans and W. H. Potts' records (1924, p. 97).

*Previous Liberian Records.*—J. Bequaert (1930, in R. P. Strong *et al.*, vol. 2, p. 994) collected in 1926 one specimen at Lenga Town (now within Harbel Plantation) and another at Memeh Town (or Memeta, on the road from Kakata to Gbanga).

*Observations During the 1943–1944 Survey.*—Adult *G. nigrofusca* were very rarely observed. During the entire time spent in Liberia, I caught only one specimen, strangely enough at Harbel Plantation. This was a male which

<sup>25</sup> There is only one record of *G. nigrofusca* from the Belgian Congo, based on an old specimen supposedly taken in 1904. I am inclined to regard it as unreliable and most probably due to an error in labelling.



was biting a native on the leg at 11 A.M., in full sunlight along an open road through a patch of light second-growth woods. The patch was surrounded by planted rubber and was a considerable distance from water. In addition the presence of this species on the Makona River (near Nyandemolahun), on the Loffa River (at Jenne), on the Kaya River (near Bolahun), and on the Lawah River (near Nikabuzu) was established by finding puparia from which a number of adults were bred.

The habits and ecological requirements of *G. nigrofusca* are as yet little known, apart from the facts that the adult fly inhabits only rain forest and fringing woods and that it will occasionally bite man. W. A. Young (1927), in the Gold Coast, and F. Zumpt (1937), in British Cameroon, recorded it as biting at night. The few adults I caught in Liberia were attacking man in broad daylight, toward noon. It is, however, possible that the normal crepuscular or nocturnal habits explain why so few of them were seen on my two trips. In the succeeding chapter on the breeding places, I contribute some notes on the puparia of this tsetse.

#### *Glossina medicorum* Austen

This species is mentioned only for the sake of completeness, as I have never observed it. It was originally described from one male and one female taken by Dr. A. McCloy, November 27, 1908, on the Sanguin River (E. E. Austen, 1911, p. 100). There is no other Liberian record. It has since been recorded from the lower Ivory Coast (E. Roubaud, 1920 and 1922; R. Newstead, A. M. Evans and W. H. Potts, 1924, p. 62), the Gold Coast, Dahomey, southern Nigeria and Prince's Island. It is unknown from Sierra Leone and French Guinea. Possibly it is a species of the coastal savanna forest in the Central and Eastern Provinces of Liberia. A. W. J. Pomeroy (1929) found the puparia in association with those of *G. palpalis* in the fringing forests of the Volta River in the Gold Coast.

#### TSETSE BREEDING GROUNDS IN LIBERIA

The species of *Glossina* are so-called pupiparous flies, owing to their unusual mode of reproduction, which they share only with a few other Diptera (Hippoboscidae, Streblidae and Nycteribiidae). The egg hatches inside the abdomen of the female fly, where it develops further in the enlarged uterus, being fed by special nursing glands. When full-grown, the last instar larva is voided by the female. It takes no further nourishment, though able to move sufficiently to bury itself in the loose material on which it is laid. Within 2 or 3 hours its skin hardens into a puparium, in which it transforms into the true pupa, producing the adult fly two or three weeks later. Only one larva develops at a time in the female, so that reproduction is very slow. It is therefore essential for the survival of the species that the gravid females larviposit in an environment suitable to further development through the pupal stage.<sup>26</sup>

<sup>26</sup> Some sticklers for formal exactitude have criticised the term "pupiparous" when applied to insects voiding full-grown larvae which pupate soon after being laid. I fail to see any serious objection to the term, since these voided larvae are potential puparia. To

The physiological requirements of the puparia vary somewhat with the species of tsetse, though seemingly not as much as those of the adults. In Liberia, for instance, I have found puparia of three species, *G. palpalis*, *G. pallicera* and *G. nigrofusca*, together under what appeared to be identical conditions. The puparia of the fourth species, *G. fusca*, were however never observed with the others. A detailed account of my findings in 1944 follows. As tsetse-fly puparia are unmistakable, much information was gathered by inducing natives to search for them for a small reward, after first showing some specimens as well as the sites where they were most likely to be found.

1. The first puparia (2 alive and 1 empty) of *G. palpalis* were seen on January 13 on the banks of the Carlo River (beyond Zowolata), a small affluent on the right bank of the St. Paul. The crossing was by dugout canoe, the river being some 250 yards wide, with a fairly broad stretch of open water between the sandy and densely wooded banks. The puparia were found in dry, loose sand, in deep shade, near overhanging roots of trees, the sand being scarcely covered with any litter. The sand was loose and dry enough for ant-lion larvae to burrow their funnel-shaped pits. Adult *G. palpalis* were also attempting to bite at this crossing.

2. At Belleyella, most of January 17 was spent looking for puparia of *G. fusca*, as the adults of this species seemed to be fairly common in this area. Although about a dozen natives helped in the search, only one breeding ground was located. This was in primary rain forest on high ground, some 2 miles from the town, and about a mile from the nearest water. A fallen tree on the side of the trail had pulled out its entire root system with the surrounding loam, thus forming a miniature cave sheltering some dry and rather loose soil. In this, one hour's search produced 10 large tsetse puparia, only one of which had not hatched. No adult fly was bred from this lot; but the characters of the puparia were those of *G. fusca* and separated them easily from those of *G. nigrofusca*, the only other large tsetse known from that section.

3. January 21 was devoted to a search for puparia on the banks of the Loffa River at Jenne. They were found without trouble in several scattered patches of loose, dry, moderately coarse sand on the rocky wooded shore. All patches were densely shaded by high trees as well as by low bushes, and usually covered with a light layer of dry leaves or litter. The puparia were at a depth of one-half to one and a half inch. The sand was so loose and dry that it passed easily through a sieve. A few adult *G. palpalis*, but no other species, were attempting to bite at the breeding places. One of the puparia had been voided shortly before, as it was yet soft and of an orange-reddish color. At the few spots which I investigated myself, about a dozen puparia per square foot was the largest number taken. In the course of the day (in about 10 hours) a total of 695 small and 15 large puparia were obtained, mostly by natives. The smaller

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call the tsetses merely "larviparous" is, moreover, misleading, as it fails to distinguish their behavior from that of the many other "larviparous" Diptera voiding larvae in the early stages (usually the first instar), which feed, grow and moult outside the body of the female. Nevertheless, I am using the verb "larviposit", which is well established in English entomological literature and involves no misunderstanding.

puparia later produced mostly *G. pallicera* and relatively few *G. palpalis*, while the larger ones only gave *G. nigrofusca*.

4. On January 22, at a small forest stream in primary forest, about a mile north of Jenne, 2 puparia of *G. palpalis* were found in a small, shaded, flat bank of loose sand, covered with dry leaves. In a similar location at another, larger forest stream, some four miles farther north, 18 puparia of both *G. palpalis* and *G. pallicera* were secured.

5. On January 23, at the crossing of the Kaia River, near Bauwolahun, 4 puparia of *G. palpalis* were obtained within a few minutes in a spot similar to the breeding places of the Loffa. During the succeeding weeks of our stay in the Bolahun region our native carriers and some of the boys of the neighboring towns brought us daily a fair number of puparia. From January 6 to February 13 a total of 4,942 puparia were obtained in this way, most of them hatching later into *G. palpalis*, a few into *G. pallicera*. There were also a few large puparia of *G. nigrofusca*. As the collecting was sporadic and no reliable record of the number of native collectors was kept, it is not possible to estimate the average number of puparia per day and per person. The first day, January 26, five boys, presumably working for about 4 hours at the Kaia River, brought back only 31 puparia. This may have been due to lack of training, as later (February 4) some 30 boys obtained 427 puparia on the same river in about 5 hours. It would seem that the wooded river banks of the Bolahun area provide, during January and February, breeding grounds as productive of *G. palpalis* puparia as any known elsewhere in tropical Africa. In Uganda, for instance, Damba Island in Lake Victoria, which supplied some 2,000 or 3,000 puparia monthly, was regarded by W. F. Fiske (1913, p. 102) as a very favorable breeding ground. The puparia from the Kaia River were bred in the hope of obtaining parasitic insects; but the incidence of parasitism was extremely low, not more than half a dozen *G. palpalis* puparia producing a minute chalcid parasite. This parasite was identified by Mr. A. B. Gahan, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, as *Syntomosphyrum glossinae* Waterston. It is a member of the family Eulophidae and has been bred from the puparia of several species of *Glossina* in different sections of Africa; but always with a very low incidence of parasitism.

6. On January 31, at the crossing of the Meio River (between Sodu and Sardu Pascia), a stream some 25 yards wide, with densely forested banks, 5 natives found 14 puparia of *G. palpalis* (2 hatched) inside 5 minutes, in a patch of rather coarse, but dry and well-shaded, bare sand, at the time some 4 to 5 feet above water level. Some adults were bred from this lot.

7. On February 2, a search in a fringing forest at the small Senja stream, 2 miles north of Foyabudu, produced within a few minutes 18 *G. palpalis* puparia in the dry sand of the bank. One of them hatched the same day.

8. On the border of French Guinea, the Mao or Makona, a river some 500 yards wide, runs mostly between steep shores, densely clothed with a narrow fringe of small trees and bushes. The banks are generally hard or covered with dried mud in which no puparia could be found. Nevertheless, on February 2, near the town of Maa, 6 natives found within an hour 47 puparia in a few patches

of loose sand, lightly covered with dry leaves. All were of *G. palpalis*; 2 hatched the same day and some later.

9. On February 15, at the forested crossing of the Kaia River near Bondualahun, 8 puparia of *G. palpalis* were found in patches of dry sand, adults of this species being fairly common also.

10. On February 23, at the Gnilia (or Ilia) River, a forest stream close to the village of Nyandemolahun, 5 *G. palpalis* puparia were found by natives in pockets of loose sand.

11. On February 24, at the ferry of the Makona River into French Guinea, a few miles from Nyandemolahun, more puparia of *G. palpalis* were found than at any other breeding place in Liberia; some 350 being brought in by about a dozen men searching for 3 to 4 hours. One large puparium of *G. nigrofusca* was also taken.

12. On March 6, on the banks of the Lawah River at Nikabuzu, 163 puparia of *G. palpalis*, *G. pallicera* and *G. nigrofusca* were obtained from densely shaded sandy patches.

The dates given above are of importance. They show that all findings were made during the driest season of the year, when the rainfall was practically nil and the streams and rivers were at their lowest level. (See the account of the climate.) After leaving me at Zorzor, Dr. Veatch returned to Bolahun and in the succeeding months made further observations on the tsetse breeding grounds in that vicinity. A specially trained native assistant, who had become very skillful in the search for puparia, was dispatched every Saturday to the banks of the Kaia and Waow Rivers, beginning in April and continuing into November. At first, when the rains were light, a fair number of puparia could be obtained, seemingly not below the average found by one person during January–February in the same locality: 65 on April 15; 70 on April 22; 45 on April 29; 105 on May 6; 40 on May 13; and 64 on May 20. Toward the end of May and during June, with the rains increasing, the numbers fell rapidly: 10 on May 29; 0 on June 3; 0 on June 10; 5 on June 17; and 2 on June 24. In July and August the rains had flooded the river banks and no puparia could be found. In September there was a short two weeks' dry spell during which a few puparia were again obtained: 5 on September 9; 0 on September 16; and 4 on September 23. Very heavy rains set in now and lasted throughout October and November, a most careful search disclosing no more puparia. As the number of puparia declined with the progress of the wet season, it was noted that adult flies became more numerous at the river banks.

In the foregoing paragraphs I have described only the localities which have actually yielded tsetse puparia. It should be emphasized, however, that in addition a most thorough and continued search in other locations was unsuccessful. Throughout the journey in the hinterland some 25 to 40 natives, in our employ as carriers, were specially instructed to search the floor of the forests and fields for small terrestrial mollusks. They were also urged to bring in any puparia of flies, a special reward being offered for these. Almost daily some dipterous puparia were brought in; but, outside those taken at the breeding grounds described above, none were of tsetse-flies. I made many similar col-

lections myself, in every conceivable environment, always with negative results.

*Breeding Requirements of G. palpalis.*<sup>27</sup>—As *G. palpalis* is the only known vector of human trypanosomiasis in Liberia, its specific breeding requirements are most important to us. Our observations show that in the hinterland the puparia of this tsetse are found exclusively in what may be called “classical breeding places”, that is, under conditions where they are known to occur in most other parts of tropical Africa (notably in Uganda and the Belgian Congo). The breeding grounds are all located along the river banks and exhibit three main characteristics. (1) Dense shade at all hours of the day, combined with proper ventilation, shelters the puparia against excessive heat. This is usually provided by plant growth some two or three feet above the surface of the soil. (2) Dry, loose, but rather coarse sand allows the newly voided larva to burrow easily to the proper depth (one-half to one and a half inches) and protects the puparium effectively against parasites and predators, as well as against moulds and mechanical disturbance. (3) Close proximity to permanent water, usually only a few feet away, insures a constant high atmospheric humidity. A combination of these special requirements prevails only in certain rather limited patches of the river banks, at what W. F. Fiske (1913, p. 104) proposed calling “favorable breeding grounds”. Here the puparia have the best chances of hatching into adult flies. The gravid female alone selects these most suitable sites. The rôle of the larva in reproduction is almost purely passive: it imbibes the secretions by the nursing glands in the body of the mother fly and, after being voided, burrows to the proper depth wherever it is dropped. In Liberia, favorable breeding grounds are more in the nature of “loci”, being small in extent and widely scattered.<sup>28</sup> Most of the river bank is either of too hard material, or too near the water level, hence too moist, or exposed to the direct rays of the sun part of the day. Moreover, conditions change continually with the seasons. These circumstances make the location of a good breeding site by the gravid fly far from easy. In addition, frequent blood meals are essential for continued reproduction. Often no or few breeding sites are available at or close to the feeding grounds, particularly at the river crossings where, as I suggested, the Liberian tsetses find perhaps their most ready diet on the natives. A proper blood diet might be unobtainable near the best breeding sites. It is evident that female *G. palpalis* must do a great deal of travelling along the river banks and cover much longer distances than the males, which usually stay close to the feeding sites, once they have discovered them. Here the males have every opportunity to mate, since the female must eventually visit the same feeding grounds.

Some of our observations suggest that in Liberia the reproductive activities

<sup>27</sup> The literature of this topic is extensive. A digest of observations prior to 1929 was published by E. Hegg (1929, pp. 485-536). For later information, consult the Review of Applied Entomology.

<sup>28</sup> There is nothing comparable in Liberia to the extensive “fly beaches”, sometimes 600 to 1,000 yards or more in length, found in Uganda on the shores of Lake Victoria (W. F. Fiske, 1920, p. 419).

of *G. palpalis* (and possibly of the other species of tsetse flies as well) may be strongly influenced by the seasons. Possibly they are carried on with success mainly, if not exclusively, during the driest months, when enough favorable breeding sites are available. We have seen that at that time many puparia can be found without much trouble. On the other hand, during the heavy rains, from July to November, the river banks are completely flooded, all low areas are waterlogged and even the forest floor on high ground is soaked. No puparia, or very few, are to be found then. A gravid female must then often be unable to locate a site answering the requirements which I have mentioned in the preceding paragraph. Possibly the reproductive activity is slowed down, or the females forsake the search for proper sites and larviposit under adverse conditions, most of the resulting puparia going to naught. This may explain perhaps why more adult flies are noticed during the wettest months, particularly at the river crossings, where they bite people, the gravid females no longer wandering away from the feeding grounds. In addition, the adult fly population is at its peak, following the many successful hatchings of the preceding drier months; while it will be at its lowest during the dry season, if few puparia were able to hatch during the foregoing wet months. Increased humidity and reduced sunshine also induce the flies to travel farther away from the river banks during the heavy rains, thus bringing them closer to the natives, particularly where the villages are near the water.<sup>29</sup> As the chances of spreading human sleeping sickness increase with the number of tsetse flies attacking people, the danger of infection may be greater during the wettest part of the year.

It should be noted, however, that while the fluctuation in level of the rivers restrains or stops breeding during the wettest months, it is not wholly detrimental to the tsetse flies. If the river level remained constant, the sandy breeding loci would eventually be covered with massed herbaceous growth or with a thick accumulation of plant litter. Either condition would render these spots unsuitable for larviposition. The periodic flooding of the banks prevents this and renovates the breeding grounds for the next season of low waters.

It is known that in the marginal areas of its range or where it attempts to survive under unsatisfactory conditions, *G. palpalis* sometimes uses unusual breeding places, which may be even several miles away from water, at least during the dry season. It is doubtful whether this is ever necessary in Liberia. It is nevertheless conceivable, though as yet not observed, that in the coastal area, close to the mangrove swamps, this tsetse may occasionally larviposit in the loose soil close to the base of oil palms (*Elaeis guineensis*) which have not been stripped of their lower petioles. Puparia commonly occur in such sites in Sierra Leone, on the Cape Lighthouse Peninsula near Freetown (W. Yorke and B. Blacklock, 1915a).

*Breeding Requirements of G. pallicra.*—The breeding grounds of this tsetse appear to have been unknown thus far. My findings show that the puparia have the same ecological requirements as those of *G. palpalis*, since both species were taken in large numbers together. As the adults of *pallicra* are not norm-

<sup>29</sup> On August 13, 1926, during the wet season, I observed several *G. palpalis* biting people in a thatched shed or "palaver house", at Lenga Town, which we were using as a camp.

ally found biting near the river banks, where the breeding sites are located, but throughout the rain forest, even sometimes several miles from water, the gravid females must travel over greater distances than those of *palpalis*. In these peregrinations the flies probably follow the trails, either cut by man or made by big animals, particularly elephants.

*Breeding Requirements of G. fusca*.—The puparia of *G. fusca* were observed by A. G. Bagshawe in Uganda, by M. Zupitza in (ex-German) Togo, and by J. Schwetz in the Belgian Congo (see E. Hegh, 1929, pp. 622–629). Schwetz' observations are particularly instructive, as he found 205 empty and 9 live puparia at a single site. This was in a very dense forest thicket, in light dry soil, beneath a layer of dead leaves, twigs and humus, at the base of the roots of a large fallen tree as well as under the tree trunk itself. It will be seen that the ecological requirements of this site were similar to those of the breeding ground I found in the primary rain forest at Belleyella, on January 17. It was a mere accident that the particular fallen tree I investigated was close to a trail. The sites are no doubt widely scattered in the forest and owing to the difficult terrain are not easy to locate.

*Breeding Requirements of G. nigrofusca*.—So far as I could trace, puparia of this tsetse had not been found previously in nature. In Liberia I obtained them in four localities, always associated with those of *G. palpalis* and in two cases also with those of *G. pallicera* (at Jenne and Nikabuzu).<sup>30</sup> The breeding grounds have therefore the ecological characteristics I described for those of *G. palpalis*.

#### LIBERIAN TSETSES AND HUMAN DISEASE: POSSIBILITIES OF CONTROL

Four species of tsetses (genus *Glossina*) are at present the only insects definitely known as *biological* vectors of the two recognized types of African human trypanosomiasis (African sleeping sickness). There is every reason to believe that the eradication of these four species in Africa would end the disease. Gambian sleeping sickness, caused by *Trypanosoma gambiense*, is the most widespread type and is transmitted mainly by *Glossina palpalis*, sometimes also by *G. tachinoides*. Rhodesian sleeping sickness, due to *Trypanosoma rhodesiense*, is restricted to parts of East Central Africa, where it is transmitted by *G. morsitans* and *G. swynnertoni*.<sup>31</sup>

Only the Gambian type of the disease occurs in the Republic of Liberia and its sole recognized vector there is *Glossina palpalis*. Efforts at controlling the disease through vector control should be directed against this tsetse only, and

<sup>30</sup> During a brief visit to Sierra Leone, Dr. Veatch, Dr. Harding and I collected 5 large tsetse puparia from a sandy site which also yielded a few puparia of *G. palpalis*. This site, on the densely forested banks of the Keya River near Balahun (between Kailahun and Bwedun), was similar to those I have described from Liberia. None of these large puparia hatched; but they were morphologically like those of Jenne from which *G. nigrofusca* was bred and I regard them as of that species.

<sup>31</sup> Other species of *Glossina* have sometimes been incriminated or suspected at least as potential vectors of the human disease, on epidemiological grounds. These claims are not at present supported by conclusive experiments. Other blood-sucking Diptera are claimed by some observers to act as *mechanical* vectors in certain areas, usually because of the alleged absence or the scarcity of tsetses. Again there is at present no conclusive evidence in support of this view.

it is the only species considered in the following discussion. Some of the other Liberian species, as well as *G. palpalis*, are no doubt potential vectors of animal trypanosomiasis; but as animal husbandry is as yet of no economic importance in Liberia, they may be neglected for the present. It is of the utmost importance in tsetse control work to realize that each of the several species of *Glossina* has its own ecological requirements, which I have attempted to bring out in the foregoing pages for the Liberian species. Control measures which have proved practical against the East African savanna species, may therefore well prove failures if applied against the riverine *G. palpalis* in the West African rain forest.

A further reservation should be made. It is now, I believe, generally recognized that any attempts at improving the Public Health of a population as a whole is primarily an economic problem. Certain methods of controlling human sleeping sickness have been successful in parts of Africa with a dense population and a well-organized economy, particularly where they were backed by the finances of a European government. It does by no means follow that the same measures could be applied in a sparsely settled country relying wholly on its own meagre resources. For this reason, I shall discuss the various methods of control directed against *G. palpalis* particularly from the point of view of Liberian conditions.<sup>32</sup>

Tsetse-flies are an instructive example of the risks attending extreme specialization. Their life economy is an intricate complex of physiological requirements which can vary only within narrow limits if the species is to survive. The equilibrium is so delicate that even a relatively slight change in the environment might upset it. It is my belief that the normal activities of mankind, in his urge at exploiting the natural resources of tropical Africa, are on the whole inimical to these insects, even if no specific measures were directed against them. In support of this view, I may cite the elimination within less than a century of *G. morsitans* from a large section of South Africa, presumably coincidental with the disappearance of the tremendous herds of game which formerly roamed over this territory; the similar fate of *G. morsitans* in certain areas of southern Katanga, where I found it very abundant in 1910-1912, but where it could no longer be detected in 1934; and the present (1944) scarcity or near-absence of *G. palpalis* at Harbel Plantation, where this tsetse was a common pest in 1926. The long-range possibility of eventually eliminating tsetse-borne diseases from at least important sections of Africa, appears rather promising.

As W. F. Fiske (1920) first stated clearly, two main factors regulate the spread of human trypanosomiasis in its entomological aspect. The first is the local density of *G. palpalis*, which depends upon three requisites: the amount of suitable food (vertebrate blood), the amount of adequate shelter, and the amount of favorable breeding grounds. The second is the contact between fly and population, that is the frequency of hungry tsetse feeding on man. In any

<sup>32</sup> I do not consider in my discussion methods of control based primarily on medical aspects of the problem, such as the complete severance of contact between the tsetse and the natives by removal of the population, or the sterilizing of the blood of disease carriers. Most of these methods seem unsuited to Liberian conditions.



area in which cases of trypanosomiasis exist or are introduced, the more flies bite the largest number of people, the greater will be the increase in the incidence of the disease. In this connection the fly's host preferences, which I discussed before, are of primary importance. I suggested the possibility of man, rather than wild vertebrates, now being the main supply of food for *G. palpalis*, at least in the more densely settled sections of Liberia and along the main trails.

*Direct Attack Upon G. palpalis, in the Adult or Pupal State.*—The most obvious method of attack, which has given spectacular results in recent years when used against other blood-sucking insect vectors, is the use of powerful insecticides, such as DDT (or gesarol) or pyrethrum and rotenone preparations. Unfortunately the feeding habits and preferred shelter of *G. palpalis* make this tsetse difficult to reach with such chemicals. The adult fly feeds in the open and does not attempt to enter walled dwellings. Only a few flies bite an individual at any one time and it would scarcely be feasible to cover the exposed skin of all natives travelling abroad with sufficient insecticide. As for spraying the forest cover of the flies, either by ground crews or from the air, the expense and labor involved would be prohibitive in Liberia, even if one were certain that the dense foliage would not effectively protect the flies. Spraying of the breeding grounds would seem to offer somewhat better possibilities, since they at least can be definitely located by finding the puparia. It would be, however, an undertaking of some magnitude, not merely because of the very many relatively small loci scattered along the numerous rivers in difficult forest country, but more so because of the need of using sufficient material to penetrate the superficial litter and some one to two inches of dirt or sand in which the puparia rest. As shown before, adult female *G. palpalis* live on the average three to four months, during which time they travel to and from the breeding grounds. A newly laid larva hatches in about 2 to 3 weeks. It would therefore be necessary to repeat the spraying of the breeding grounds at least every fortnight over several months. Moreover, the method could hope to succeed permanently only if no breeding grounds were missed. Insecticide spraying against tsetses has, so far as I know, not been attempted anywhere in tropical Africa and might well be worth experimenting with, particularly in the marginal areas of *G. palpalis*.

Destruction of adult tsetses by hand catching and trapping is often advocated. It seems to have been particularly successful against certain savanna tsetses under special environmental conditions. Its value against *G. palpalis* in the West African rain forest is rather dubious. In Liberia hand catching by natives would have to be stimulated by some bounty, and it is doubtful whether such a system could reduce the fly population sufficiently to have an effect upon the rate of infection with human trypanosomiasis. The results of trapping *G. palpalis* are much disputed and most workers who attempted it have felt that it could not eradicate this species. L. Van Hoof, C. Henrard and E. Peel (1938) concluded from their experiments in the Belgian Congo that properly trained fly boys could catch more flies in the same time than the traps, which, moreover, required a large and competent personnel. Destruction by tangle-

foot or other types of glue are handicapped by the failure of *G. palpalis* to settle commonly on the back of people where the sticky surface must of necessity be carried.

Attempts to attract the gravid female to artificial breeding sites, where the puparia would be concentrated and then regularly destroyed, were carried on with some success in Uganda by G. D. H. Carpenter (1923). The method calls for a trained personnel and constant supervision and is scarcely suitable to Liberian conditions. E. Hegh (1930) discusses various other suggestions for the control of the puparia.

*Ecological Control.*—In view of the difficulties of controlling *G. palpalis* in Liberia by directly attacking either the adult or the puparium, ecological methods are most likely to yield results commensurate with the effort. Such methods aim at modifying the environment so as to make it unsuitable to the insect. Adult *G. palpalis* require a peculiar type of cover (well-shaded massive undergrowth in the woody fringes of rivers, with an open stretch of water between the banks) and a readily accessible supply of the proper blood diet (in Liberia primarily crocodiles and monitors and, in their absence, man). The puparia of this tsetse on the other hand need a special type of breeding grounds (loose, dry, well-shaded sand or dirt, close to, but beyond the reach of permanent water).

Selective clearing is the most practical way of altering the environment so as to render it unfit to the adult and the puparium. Removal of the woody cover from the banks, to a depth of at most a quarter mile from the water's edge, will effectively clear the river of *G. palpalis*. Obviously, this cannot be carried out over the entire infected course of all the rivers in the continuous rain forest area which covers the major part of Liberia. Clearing should here be limited to the vicinity of river crossings (bridges, fords, and ferries), beginning with those on the main trails which are used daily by natives travelling over long distances. The destruction of the bushes and low trees should be complete, although the very large trees may be left standing. The cleared ground should be kept free of new woody growth, the best procedure for lasting results being to plant some low-growing crop, such as sweet potatoes or peanuts on higher ground, or swamp rice in the lower-lying spots. The clearing should extend on both banks over at least a quarter mile and preferably over a half mile above and below the crossing. It is the clearing of the river banks which has freed much of Harbel Plantation of *G. palpalis*. If it were completed on the few remaining wooded stretches of the Farmington and Du Rivers and their affluents, the tsetse would be completely eliminated from this locality.

In the rather small area covered with savanna in northwestern Liberia, it might be possible without too much effort to eradicate *G. palpalis*. In this section the forest fringes of the rivers, haunted by the flies and sheltering the breeding places, are comparatively narrow, often only a few hundred yards wide. They could be readily cut down by sections, first close to the river crossings and eventually over the whole length of the rivers. As this is a region of relatively dense population, but with a rather high infection rate, I suggest

that, if preventive control measures were to be undertaken in Liberia, they should be attempted first in this area.

Although *G. palpalis* has many predatory enemies and several parasites, it is extremely doubtful whether any of these destructors could be put to practical use. W. F. Fiske (1920, p. 436) pointed out that they are manifestly unable to prevent the tsetse flies from surviving in numbers sufficient for disease transmission, in areas where the other ecological conditions are favorable to both the adult fly and the puparium. As these other conditions also protect the tsetse against the destructors, it would seem more effective to make the environment itself unsuitable to the insect.

*Individual Protection.*—While the usual protective clothing recommended against blood-sucking Diptera in the tropics may be of some help, it should be noted that tsetse flies readily bite even through relatively thick woven material. A muslin headgear worn over the face and neck is not of particular value against *G. palpalis*, as this tsetse prefers to attack the lower extremities (ankles and inside the trouser legs). If a headgear be used, the part before the face should be of a black material, which color impairs vision much less than white. Leggings are a good protection, particularly while crossing rivers or travelling in canoes.

Various repellents have been tried against tsetse flies, particularly against the species attacking domestic animals, but none were found satisfactory. H. E. Hornby and M. H. French (1943), after experimenting with a variety of substances, concluded that only pyrethrum or preparations of pyrethrin gave any promise of practical efficacy. Even for these, however, it is far from certain that an infected tsetse may not probe the skin and inject infective trypanosomes before it is poisoned or paralyzed by the repellent. J. R. Holden and G. M. Findlay (1944), in the Gold Coast, tried a repellent consisting of pyrethrum in a vanishing-cream base, against *Glossina palpalis*. In the laboratory they found that, if given a choice, the flies failed to bite on the arm treated with the cream, though they bit on the untreated arm. In nature it was observed that about the same number of *palpalis* settled on anointed natives as on the unanointed controls; but the ointment prevents biting up to at least six hours after the application, provided no sweating occurs. The repellent action was, however, destroyed rapidly by profuse sweating, particularly in association with exposure to the sun, which makes it of relatively little practical use under most circumstances.

*Tsetse-Flies and Air Transport.*—Fisherman Lake in Liberia is at present the chief terminal for airplane travel between Africa and Brazil. The transatlantic flights are by hydroplanes which stop and take off on the waters of the Lake about half a mile from the shore, where accommodations for resident officials and transients are available near a small airfield. Originally the tidal waters of the Lake were fringed with mangrove swamp. This has been cleared away at the airfield landing place, but the clearing should be extended much farther to be effective against *G. palpalis* (at least a mile in each direction from the landing pier). That tsetse flies board transatlantic planes in this locality is proved by their being found at Natal on two occasions in 1941 and 1942 after disin-

sectization (F. L. Soper and D. B. Wilson, 1943, pp. 139 and 228). Even less clearing of the mangrove has been carried on near the landing place of the planes in the lagoon of Monrovia. The large airfield near Harbel Plantation, now operated by the U. S. Army, is so completely cleared that no *G. palpalis* is likely to board a plane there. As pointed out by F. L. Soper and D. B. Wilson (1943), the problem of preventing the transport of disease vectors by air travel is a most serious one. In the case of tsetse-flies it is impossible to predict whether or not these insects could successfully spread in the New World, where they do not live at present. If they became established, however, they would sooner or later become the vectors not only of human, but also of animal types of trypanosomiasis. The economic danger of their importation is perhaps greater than that to public health. If it were found that the airports of Liberia cannot be adequately protected against tsetse-flies, it might be wiser for the Brazilian authorities to forbid all air travel between the two countries.

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## PART III

# THE TREATMENT OF AFRICAN SLEEPING SICKNESS WITH TWO NEW TRIVALENT ARSENICAL PREPARATIONS (MELARSEN OXIDE AND 70A)<sup>2, 3, 4</sup>

BY DAVID WEINMAN, M.D.<sup>1</sup>

### INTRODUCTION

The treatment of African trypanosomiasis has not been bettered in some years. The therapy remains what it has been, chief reliance being placed on tryparsamide and on Bayer 205 (= germanin = antrypol = naphuride). These compounds, remarkably effective as they are, do have certain defects, and to substitute for them improved chemotherapeutic agents would constitute a decided advance.

The improvements to be sought are: lowered toxicity, a higher degree of effectiveness, a wider range of action to include infections with *T. rhodesiense*, greater ease of administration and reduced time of treatment.

The two trivalent arsenical compounds reported on herein have, in experimental animal trypanosomiasis, distinctly higher chemotherapeutic indices than tryparsamide. It remained to be seen whether this suggestion of increased effectiveness would prove to apply to human infections also.

### GENERAL PROCEDURE, SELECTION OF PATIENTS, NATURE OF THE DISEASE, ETC.

These studies were made in collaboration with Dr. Karl Franz at the Hospital of the Firestone Plantations Company, Harbel, Liberia.<sup>5</sup> The patients were all native laborers on the plantation, or members of their families.

As the accompanying map shows, cases of trypanosomiasis treated at the hospital were, for the most part, not of local origin, but came from different parts of Liberia where presumably the disease is established and the patients contracted their infection. On the Harbel plantation it may be considered that transmission does not take place and accordingly that in our results no heed need be paid to the possibility of reinfection of our patients.

<sup>1</sup> In collaboration with Karl Franz, M.D.

<sup>2</sup> From the Department of Comparative Pathology and Tropical Medicine, Harvard Schools of Medicine and Public Health, Boston, Massachusetts.

<sup>3</sup> Publication No. 3 of the Harvard Liberian Expedition under the joint auspices of Harvard University and The American Foundation for Tropical Medicine, Inc.

<sup>4</sup> A preliminary report was read November 25, 1944, before the American Society of Tropical Medicine, and appeared in The American Journal of Tropical Medicine, 1945, 25, 343-344.

<sup>5</sup> Hospital space and laboratory facilities were very kindly made available through the courtesy of: Mr. Byron H. Larabee, Vice-President, Firestone Plantations Company, Mr. Ross E. Wilson, General Manager, Firestone Plantations, Harbel, and Dr. James L. Doenges, Chief Surgeon, Firestone Plantations Hospital, Harbel.



that there has never been a case of sleeping sickness in the imported non-African staff during their residence on the plantation.<sup>6</sup>

Suspects were selected because of enlarged cervical lymph nodes or suggestive complaints, particularly somnolence or insomnia. These suspects were sent to the hospital where a lymph node puncture was performed and a thick drop of the blood made and stained. If either of these was positive or if there were any signs or symptoms of central nervous system involvement, a lumbar puncture was made and in the spinal fluid the cells were counted and the centrifuged sediment examined for trypanosomes. Thus, all positive cases had a spinal fluid examination before treatment was instituted.

Patients were hospitalized during treatment, and examined and interrogated for evidence of toxic reactions. The weight was carefully watched. Blood and urine examinations were routinely performed for evidence of hematopoietic or renal damage. At the end of treatment the blood was re-examined for trypanosomes, also the lymph nodes if puncturable. A second lumbar puncture was performed on all patients with abnormal spinal fluids. When discharged all patients were instructed to return at monthly intervals for re-examination and a monetary inducement offered to make their return more probable.

In general, the patients were underweight, undernourished, and probably suffered from various dietary deficiencies, notably a lack of protein. In addition, almost all suffered from some disease other than sleeping sickness: yaws was reported to be almost universal, some had schistosomiasis, and many a chronic pruriginous dermatitis (? sarcoptic crawl-crawl). In general, they seemed poor physical risks and this influenced our decision to use small intravenous doses of Melarsen oxide in this first trial in human beings.

As to the nature of the disease in Liberia, it appears to be of a mild type, responding well to moderate doses of tryparsamide (10 to 16-18 grams) and no "arsenic-resistant" cases had been encountered at the Plantation hospital in years for which data was available.<sup>7</sup> In this connection, it may be pointed out that systematic treatment of trypanosomiasis is of relatively recent date in Liberia, probably within the last fifteen years (10), and certainly not longer than twenty years, when the first proved cases were described by Dr. Max Theiler of the 1926 Harvard Expedition (13).

#### MELARSEN OXIDE<sup>8</sup>

##### *Chemistry and Physics*

Melarsen oxide is a white crystalline substance containing 22.8% arsenic in the trivalent form. It is very slightly soluble in cold water and soluble to about

<sup>6</sup> A personal communication from Dr. J. L. Doenges, formerly Chief Surgeon, Firestone Plantations Hospital, Harbel, Liberia, states: "During the two years that I have been here no member of the resident white staff has been found to have contracted sleeping sickness. Furthermore, there is no record in the hospital files of a case of sleeping sickness in a white man residing on the Harbel plantation."

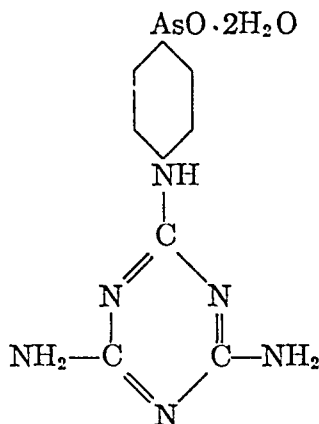
<sup>7</sup> Personal communication from Dr. Franz.

<sup>8</sup> The Melarsen oxide for clinical study was very kindly furnished by Parke, Davis and Company.



1.0% in boiling water. In propylene glycol, 5% solutions may be obtained and advantage is taken of this fact to obtain injectable preparations, solution first being obtained in pure propylene glycol to which distilled water may subsequently be added (1, 2).

The structural formula is:



It is referred to as 2-(4'-arsenosooanilino)-4,6-diamino-s-triazine dihydrate, also as *p*-(2,4-Diamino-s-triazinyl-6)-aminophenyl arsine oxide. The empirical formula is  $C_9H_9AsN_6O \cdot 2H_2O$  (1, 2, 6).

Melarsen and its oxide are compounds synthesized by E. A. H. Friedheim in an effort to develop potent trypanocidal agents of reduced toxicity for the central nervous system, particularly the optic nerve. Clinical trials of Melarsen with the designation 4289 or "acide triazine-arsinique" were distinctly favorable (8).

The oxide is closely allied to the pentavalent Melarsen from which it is prepared by the reduction of the arsenic present as arsonic acid in Melarsen to the corresponding arsine oxide derivative.

The oxide is stable; no deterioration of the solid chemical was noted in two and a half years of observation, and the solutions may be expected to be stable for at least four to five years at tropical temperatures. It is not stable at autoclave temperature and sterile solutions are obtained by filtration (1).

### Pharmacology

*Curative Effect.*—This was tested in rats against *Trypanosoma equiperdum* infections. The average single intravenous minimal curative dose (M.C.D.) is 0.50 milligrams per kilogram. This is defined as the minimal dose which, in heavily infected rats, causes disappearance of the trypanosomes during an observation period of 4 weeks. The LD<sub>50</sub> dose being 17.5 mg./kg., the curative index  $\frac{LD_{50}}{M.C.D.}$  is 35 which compares very favorably with the curative index of 4 for trypanamide under the same conditions. A minimal therapeutic dose (M.Th.D.) had a temporary effect with as little as 0.10 mg./kg. The thera-

peutic index (Th.I.) is the ratio  $\frac{LD50}{M.Th.D}$  and for Melarsen oxide is 175 whereas for tryparsamide it is 20 (2, 9).

A comparison of Melarsen oxide and tryparsamide is given in Table I.

Different animals show some variations in regard to the intravenous dose of Melarsen oxide causing toxic reactions. In rabbits the LD50 is about 12 mg./kg.; whereas dogs do not survive with 4 mg./kg. but do withstand 2.0 mg./kg. All rats survive a dose of 7.5 mg./kg. given intravenously or subcutaneously. The corresponding LD50 doses are 17.5 and 39.5 mg./kg. (9).

Orally, Melarsen oxide is absorbed from the gastro-intestinal tract. The oral minimal curative dose against *T. equiperdum* infections in rats is 7.5 mg./kg. or 1/93rd of the dose which 100% of rats withstand, since they all survive 700 mg./kg. The LD50 is 950 mg./kg. All mice survive 0.75 mg./mouse (approximately 37.5 mg./kg.), the LD50 is about 53.5 mg./kg.<sup>9</sup> Rabbits withstand relatively large doses, 4 out of 5 survived 100 mg./kg. and 3 out of 5, 150mg./kg. (9).

TABLE I

*Comparison of Melarsen oxide and Tryparsamide*

Single intravenous doses expressed in milligrams of drug per kilogram of weight;  
*Trypanosoma equiperdum* infection in rats

	LD50	M.Th.D.	M.C.D.	Th.I.	C.I.
Melarsen oxide.....	17.5	0.10	0.50	175	35
Tryparsamide.....	4000	200	1000	20	4*

\* Dr. Louise Pearce states that C.I. of tryparsamide is 3 (11).

Chronic oral toxicity has been studied in dogs. There was some initial weight loss in many cases, the weight later becoming stabilized or increasing. With doses of 3 to 10 mg./kg. to total 64 doses in 72 days occasional vomiting occurred in the first 10 days. No other untoward reactions were observed.

Higher doses were followed by more frequent vomiting. Dogs receiving 20 mg./kg. for 60 doses in 80 days showed slight anemia. With a dose of 30 mg./kg. daily, although no acute reaction took place, the dogs lost considerable weight, developed ulcerative stomatitis and death occurred after 38 doses in 60 days (9).

*Distribution in tissues.*—A concentration in the brain of 4.9 micrograms of arsenic/100 gm. of brain tissue (= 0.214 mg. of Melarsen oxide/kg.) was recorded for a dog which had received 0.25 mg./kg. intravenously daily for 21 doses in 29 days. The blood concentration at the time was only one-third as much. Concentrations in the heart and muscle were about the same as in the brain, they were 5 to 10 times greater in the spleen and liver and 30 times as much in the kidneys (9).

<sup>9</sup> Calculated on a basis of 20 gm. per mouse.

*Posology*

As we have stated previously our patients were in poor physical condition, underweight, apparently suffering from malnutrition as well as from various illnesses other than trypanosomiasis.

This influenced our choice in dosage, *primum non nocere* seeming a particularly valid principle in the first trial of a new drug on human beings. Accordingly, the minimal therapeutic dose of 0.10 mg./kg. was chosen for intravenous therapy. This dose is 1/175th of the LD50 for rats, 1/100th of that at which 90% of rats survive and 1/75th of the dose giving 100% survival. It is to be remarked here and will be emphasized later that this dose may be considerably lower than an equally safe dose giving maximal effect.

TABLE II  
Toxicity Table (in Mg. of Drug per Kg. of Weight (9))

	INTRAVENOUS			ORAL	
	Animal	100% survive	LD50	100% survive	LD50
Acute (single doses)	Rat	7.5 mg.	17.5 mg.	700 mg.	950 mg.
	Rabbit	6.0 mg.	12.0 mg.	80 mg.	160 mg.
	Dog	2.0 mg.	3.0 mg.	60+ mg.	—
	Mouse	7.5 mg.	17.5 mg.	37.5 mg.	53.5 mg.
Chronic (repeated doses)	Dog	0.5 mg./kg. daily for 21 doses in 29 days	—	20 mg./kg. for 60 doses in 80 days  60 mg./kg. for 8 doses in 10 days*	30 mg./kg. for 38 doses in 60 days  —
	Rabbit			3.0 mg./kg. for 9 doses in 3 days	6.5 mg./kg. for 9 doses in 13 days

\* Vomiting occurred 1 to 4 hours after treatment. The amount of drug retained is questionable.

The dose chosen (0.10 mg./kg.) was given daily for 7 injections as the rule; this schedule being adopted in order to determine whether with Melarsen oxide it would be possible to complete the therapy in one week. The drug was provided in sterile ampoules dissolved in propylene glycol, the concentration being such that the amount injected was less than 0.30 cc. The oral dose adopted was 3.0 mg./kg. This was given for 5 to 8 days. Three of these patients were then given a course of intravenous Melarsen oxide as described above. For oral use the drug was provided in gelatine capsules.

A total of 18 patients were made available for this treatment. Three of these received both oral and intravenous therapy. After termination of the course of treatment the patients were followed as long as possible. Unfortunately,

intellectual persuasion, gratuitous therapy, free board and lodging and cash rewards induced only a limited number to return for re-examination.

### *Oral Administration of Melarsen Oxide*

Administered in gelatine capsules, the compound was given at a dose of 3.0 mg./kg. daily for a period of 5 to 8 days for a single course. No toxic reactions were noted; the weight remained stable or increased. The maximal dose given was 1440 mg. in 8 days.

1) *Non-neurological cases*.—Four patients were found to have trypanosomes in the blood or lymph nodes, but not in the spinal fluid which contained a normal amount of cells. After treatment all patients were negative. One was seen at 60 days; his blood and lymph nodes were negative; he did not return at a later date for a spinal tap. The 3 others were seen 7 months, 8 months and one year after treatment. The spinal fluids were normal, blood and lymph nodes showed no trypanosomes.

2) *Neurological cases*.—Eight cases showed from 65 to 1200 cells in the spinal fluid, which in four instances also contained trypanosomes. Six also had positive blood or lymph nodes. Two cases had received prior treatment with 70A ( $p\text{-(CH}_2)_3\text{COOH}$  phenyl arsenoxide).

The dose given was the same, 3.0 mg./kg. for 5 to 8 days. There was immediate benefit apparent at the end of the course in 6 of the 8 cases as adjudged by the fall in the cell count and disappearance of trypanosomes from the spinal fluid. The 2 resistant cases showed an equally favorable response after a second oral course.

Four of these patients were in the group 250 cells or more. These had an average of 603 cells which was reduced to 210 after the first course and to 105 cells after the second course. The other 4 had from 65–148 cells with an average of 108. These were reduced to 42 after the first course. Prior treatment with 70A did not seem to exert any adverse effect.

Of the 4 more advanced cases (*i.e.* 250 cells or more), 2 maintained their improvement as long as they were followed, 2 others relapsed. These last had each been given a first course lasting 7 days and a second one of 5 days' duration. One was subsequently given a course of intravenous Melarsen oxide without benefit. Both were ultimately given 18 gms. of tryparsamide (the usual total dose in Liberia) which reduced the cell count to the neighborhood of or below that attained by Melarsen oxide; it is not yet known whether this improvement was maintained.

### *Intravenous Melarsen Oxide*

The drug was given intravenously to 9 patients at a dose of 0.10 mg./kg. for 7 doses on consecutive days. The highest total dose given was 46.2 mg. in 7 days. One patient left the hospital without authorization after 3 doses. Another received 9 doses in all, but a pneumonia which developed after the second injection caused an interruption of treatment for 2 weeks, following which 7 consecutive daily doses were given.

1) *Non-neurological cases*.—This group comprised 5 patients. At the end of the period of intravenous treatment, blood and lymph node examinations of all were negative. Likewise, 3 of these patients re-examined at 30, 30 and 60 days were also negative. This group did not cooperate by returning for repeated re-examinations, possibly because they did not feel indisposed. However, these data may be completed by reference to the similar group treated with oral Melarsen oxide and where no relapses were noted in periods running over 6 months (*vide supra*).

2) *Neurological cases*.—These comprised 4 patients, 3 of whom had received prior treatment with arsenical compounds. Of these 3 one was distinctly benefitted—the cell count falling from 110 to 9 where it remained one month after cessation of treatment. Of the other 2 previously treated patients one advanced case showed a relapse with an increase in cells 35 days after treatment; the other was not aided. The fourth case left the hospital without authorization after three injections.

### *Clinical Response*

In general, there was an improvement in the patients which paralleled the objective findings. Headaches, body aches and pains and somnolence usually disappeared or were markedly diminished, whereas characteristic signs of the cerebral form did not respond so readily. Tremors, ankle clonus, hyperactive reflexes and Romberg's sign were usually diminished and sometimes, but by no means always, disappeared completely.

### *Toxicity*

Melarsen oxide was remarkably well tolerated and at the doses given no toxic reactions were noted. A few patients passed urine containing small amounts of protein but this did not increase with continuation of the drug. There was no loss of appetite nor loss of weight following oral therapy. Red cell totals did not vary appreciably from the pre-treatment levels. There were no visual disturbances in any of our patients. No Herxheimer type reactions occurred in any of our cerebral cases.

### *Summary and Discussion of Results with Melarsen Oxide*

It seems clear that in Melarsen oxide we have a trivalent arsenical compound which is active in the cerebral stage as well as the earlier stages of African trypanosomiasis due to *T. gambiense*.

The doses employed seem adequate for the treatment of non-neurological cases. All such patients, 9 in number, became negative at the end of treatment, whether treated intravenously or orally. In no case was there a relapse. In 3 of these patients the cerebrospinal fluid was examined 6 months to one year after the end of treatment and was found to be normal.

The neurological cases were more difficult to treat as the universal experience of others had led us to anticipate. We used the same doses as for the early stages and observed immediate amelioration in all 9 cases, apparent cures in 2,

relapses in 2 others, while the majority—5—were clearly benefited but did not show normal spinal fluids at the end of the observation period. For comparison, tryparsamide is said to give 58.7% apparent successes (range 15.8 to 82.9%), 24.7% improved, and 16.6% failures (range 7.2 to 50%) in advanced cases, advanced being defined as with more than 10 cells in the cerebrospinal fluid (11). As to the relative advantages of oral and parenteral administration, our series is too small to permit of conclusion; however, one case showed only a mediocre response to oral therapy but was apparently cured by a single intravenous course.

It seems likely that, in this first trial of Melarsen oxide in man, we did not utilize a dosage producing optimal effects in the cerebral cases. Accordingly, improved results may subsequently be obtained by varying the individual or total doses, rhythm of injection and possibly other factors. We do feel that our present results warrant further study of the compound to determine this optimum and then a detailed comparison with drugs in current use in regard to cure rates and toxic reactions.

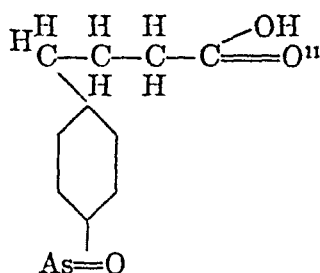
No *Trypanosoma rhodesiense*-infected patients were available for treatment. However, in animals so inoculated with *T. rhodesiense* as to produce cerebral trypanosomiasis, the compound has proved to be curative.<sup>10</sup>

### 70A

The second drug employed was also a trivalent arsenical furnished under the designation "70A".

#### *Chemistry, Physics and Pharmacology*

70A is p-(CH<sub>2</sub>)<sub>3</sub> COOH phenyl arsenoxide. The compound referred to as γ-(P-Arsenosophenyl)-butyric acid is identical with it and all have the structural formula



In acute *T. equiperdum* infections in mice, 70A was about 5 times as effective as tryparsamide; in chronic infections in rabbits, about twice as active.

The drug was furnished<sup>12</sup> in sterile ampoules containing 40 mg. in 2.0 cc. All injections were made intravenously and following one or two trial half-doses, the amount given was always 2.0 cc. (= 40 mg.). This was administered thrice

<sup>10</sup> These experiments will be reported elsewhere.

<sup>11</sup> According to Dr. Gertrude Spremulli who very kindly vouched for the identity of these compounds.

<sup>12</sup> Dr. Harry Eagle very kindly supplied the 70A for these trials.

weekly to total between 360 and 400 mg., except in one child where the dosage was adjusted in proportion to weight.

Ten patients were so treated. In all of these the blood and lymph nodes became negative at the end of the course. Four of these patients have been followed for periods of 2½, 5, 9 and 11 months, and none has relapsed.

In regard to the cerebral cases, 3 in number, 70A was ineffectual. These cases received from 380 to 400 mg., despite which the spinal fluid cell counts at the end of treatment were higher than at the start, the average rising from 8 cells prior to treatment to 93 cells one week to 9 months afterwards. One patient had an initial count of 14 cells and no trypanosomes in the spinal fluid; 6 days after receiving a total of 380 mg., trypanosomes were present and the cells numbered 148.

Although the number of cases treated was small, it seems clear that at the doses employed, 70A was effective in the early stage but neither prevented nor ameliorated cerebral involvement.<sup>13</sup>

#### SUMMARY AND CONCLUSIONS

1. Two new trivalent organic arsenicals with favorable chemotherapeutic indices as compared with tryparsamide were given trial against African sleeping sickness caused by *Trypanosoma gambiense*.

2. One of these trivalent compounds, Melarsen oxide, is not only active in the blood and lymph node stage but also has a pronounced effect in the cerebral stage.

3. These effects followed both oral and intravenous administration.

4. No toxic reactions to the doses of Melarsen oxide were noted in any of our patients.

5. In the early stage, the dosage of Melarsen oxide was adequate to produce uniformly satisfactory results. The same dosage given to patients with cerebral involvement always produced amelioration, but this improvement was sometimes only temporary. It seems quite probable that in this initial trial the single dosage and rhythm of injection adopted was not the optimal therapy for advanced patients.

6. The results thus far obtained with Melarsen oxide appear sufficiently encouraging to warrant further investigation in order to determine this optimum and then to compare the compound in regard to cure and relapse rates, toxicity and also activity in *Trypanosoma rhodesiense* infections with drugs now in current use.

7. The second drug employed, 70A, appears to be effective in the early stage of gambian trypanosomiasis, but neither prevented nor ameliorated cerebral involvement despite intravenous doses of 380 to 400 mg. administered during 20 to 30 days.

<sup>13</sup> Dr. Harry Eagle has recently reported on this compound (4); he states that results in late cases are "not encouraging" and suggests combined treatment with a pentavalent arsenical.

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# AN EPIDEMIOLOGICAL STUDY OF JUNGLE YELLOW FEVER IN AN ENDEMIC AREA IN BRAZIL\*

## PART I—EPIDEMIOLOGY OF HUMAN INFECTIONS

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## PART II—INVESTIGATIONS OF VERTEBRATE HOSTS AND ARTHROPOD VECTORS

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## PART I. EPIDEMIOLOGY OF HUMAN INFECTIONS

The problem of jungle yellow fever in Brazil becomes relatively more important with the effective control of *Aedes aegypti* in all urban centers and its eradication from large sections of the country. Indeed, among the 1,258 liver specimens collected by the Viscerotomy Service from January of 1932 to June of 1945, on which the diagnosis of yellow fever was made, only 78 came from localities where *A. aegypti* still existed. The remainder (1,180) were derived from rural areas where, presumably, the infection was acquired through the bite of some forest-breeding mosquito. Moreover, this epidemiological variety of the disease is not only significant in being responsible for an overwhelming proportion of human infections in recent years, but also in constituting uncontrolled foci of the virus.

The transmission of yellow fever by sylvan mosquitoes was suspected by Lutz (1) in 1929, but convincing proof that human infection may occur in the absence of *A. aegypti* was not forthcoming until a few years later when Soper *et al* (2) investigated a rural epidemic in Valle do Chanaan, State of Espirito Santo. The virus isolated from one of the human infections during this rural epidemic was shown to be immunologically identical with the virus of classic urban yellow fever transmitted by *A. aegypti*. Thereafter, additional rural outbreaks were investigated and reported (Burke (3) and Aragão (4)), and the term "jungle"<sup>1</sup> yellow fever (5, 6) was applied to distinguish its epidemiological features from the urban *A. aegypti*-transmitted pattern of the disease.

\*The work on which these observations are based was done under the auspices of the Serviço de Estudos e Pesquisas sobre a Febre Amarela (Yellow Fever Research Service), which is maintained jointly by the Ministry of Education and Health of Brazil, and the International Health Division of The Rockefeller Foundation.

<sup>1</sup>The term "selvatic", "sylvatic" or "sylvan" yellow fever would be more in keeping with "sylvestre" and "silvestre" used in Spanish and Portuguese, but the designation "jungle" yellow fever has been adhered to in this paper in deference to priority and because a change to another name, even though more suitable, would likely lead to confusion. Also, as pointed out by Soper (Clinical and Tropical Medicine p. 396) human infection is usually associated with contact with a type of dense forest growth which commonly warrants the application of the more restrictive English term of jungle. That is, forest so dense as to preclude largely the penetration of sunlight to the ground.

Since there is no immunological difference between yellow fever virus strains of jungle and of urban origin, it is not surprising that urban epidemics should arise from the introduction of the jungle virus into urban centers harboring *A. aegypti*. Several such instances have been reported (Walcott *et al* (7) and Soper (8)). Indeed, the last known occurrence of *A. aegypti*-transmitted yellow fever in Brazil took place in the town of Senna Madureira, situated on the Iaco River in the Amazon basin, Acre Territory, and was undoubtedly attributable to the entrance of the virus from the neighboring forests (Silveira and Simas (9)). In that yellow fever virus is no longer being maintained by the man-*A. aegypti* cycle on this continent, it can be postulated that any future urban epidemics of neotropical origin will result from the introduction of a jungle strain.

The outbreak in Matto Grosso in 1934-1935 described by Burke (3), initiated an epidemic wave which spread eastward and southward, and during the succeeding five years engulfed the major part of southern Brazil. In this instance the term epidemic may be justifiably applied because the virus appeared to "burn itself out" as it progressed and rarely lasted longer than one season in any given locality. Finally it retreated in a northeasterly direction and disappeared, and for a period of three years no recognized yellow fever occurred south of the Amazon watershed and the humid rain-forest portion of southern Bahia. The full history and details of this epidemic are yet to be published, but Soper (8, 10, 11) in reviewing the general aspects of jungle yellow fever, includes the results of several localized studies made during the course of this epidemic. It may be added that almost exactly 10 years after the large last outbreak, yellow fever again appeared (December 1943) in Matto Grosso, and 12 months later human infections occurred in the vicinity of the town of Goyaz in the State of Goyaz. During the ensuing five months the infection spread southward and eastward through the southern part of the State of Goyaz into the State of Minas Geraes, at one point approximating the northern border of the State of São Paulo. Thus, the course and progress of the former epidemic which began in 1934 has been duplicated, so far, with singular fidelity.

On the other hand, the continued periodic collection of yellow fever-positive livers, although small in number, coupled with the results of immunity surveys (Soper (12)), implies that jungle yellow fever exists in endemic form in the densely forested portions of the Amazon valley and in the southern part of the State of Bahia. It may be inferred, therefore, that jungle yellow fever occurs in the southern and more or less subtropical parts of Brazil in the form of periodic epidemics, while to the north in the humid tropical forests it is endemic in character. The occurrence of human infections in the epidemic zone, besides being transient in nature, is definitely seasonal in distribution and is limited almost exclusively to the warmer rainy season from November to May, while in the endemic zone it may take place at any season of the year.

Hitherto, intensive epidemiological investigations of jungle yellow fever in Brazil have been confined largely to transient or epidemic outbreaks. These epidemic waves in southern Brazil have accounted for a large majority of the known human infections during the past 10 years, and their manner of spread is

still puzzling. The transient nature of these waves precludes a prolonged study of animal hosts and arthropod vectors, as well as the circumstances that contribute to infection of humans. Moreover, since it is presumed that the epidemics owe their origin to "spill-overs" of the virus from the northern endemic areas, a more complete knowledge of the propagation and maintenance of the virus in endemic zones should be helpful in elucidating the cause of the recurring epidemics.

The studies here reported were made in the vicinity of Ilhéus, a town located on the Atlantic seaboard of the State of Bahia, where rural yellow fever, in the absence of *A. aegypti*, had been recognized at intervals during the preceding 10 years.

The foregoing review of the literature has been limited to investigations in Brazil especially related to human infections. A review of publications in reference to animal hosts and arthropod vectors will be given later.

*History of Yellow Fever in the Ilhéus Region:* Reliable information on the occurrence of yellow fever and the distribution of *A. aegypti* in the Ilhéus region dates from 1930 when a post of the Cooperative Yellow Fever Service was established in the town of Ilhéus (map 1). During the preceding 30 years yellow fever was more or less continuously present in the State of Bahia in the vicinity of Salvador. While exact data are lacking, it is highly probable that the port of Ilhéus and inland towns along the railroad were subjected to periodic visitations of *A. aegypti*-transmitted epidemics.

A survey made in 1930 verified the presence of *A. aegypti* in the town of Ilhéus and in a number of other towns situated on or near the railroad and the main highway between Ilhéus and Itabuna. It is of interest to note that this mosquito never penetrated to the strictly rural areas nor did it reach villages off the principal lines of communication. By 1934 the control measures, which were instituted in 1930, had been successful in eradicating *A. aegypti* from most of the centers where it had formerly existed. It was still present, although the index was low, in Castelo Novo, Ferradas, Itabuna, Buerarema and Sambaituba. By 1936 *A. aegypti* persisted only in the town of Castelo Novo but during the following year this smoldering focus was extinguished, thus eradicating this mosquito from the entire region. Thereafter continued searches have failed to establish its presence.

Viscerotomy posts for collecting liver specimens were inaugurated in 1931, and during the succeeding year enough posts were functioning to assure fairly complete coverage of the *municípios*<sup>2</sup> (counties) of Ilhéus and Itabuna.

The last suspected case of yellow fever in the town of Ilhéus occurred in 1933. The victim was a male who several days prior to onset of illness had gone hunting in a neighboring forest. From what is now known it is highly probable that he was infected by sylvan mosquitoes during the hunting trip. Although the man died, no liver specimen was obtained.

Following the creation of viscerotomy posts, the first liver specimen indicating yellow fever infection was obtained in 1934. It came from a child three years of

<sup>2</sup> A *município* is analogous to a county in the United States and embraces both rural and urban areas.

age who had died on May 30th of that year after an illness of four days. The child lived in an isolated, densely wooded locality known as Sta. Rita in the district of Japu, Ilhéus. An epidemiological investigation made by Dr. Virgilio de Oliveira, Chief of the Yellow Fever Control Service in Ilhéus, and Dr. Paulo C. A. Antunes of the National Yellow Fever Service, revealed that infection

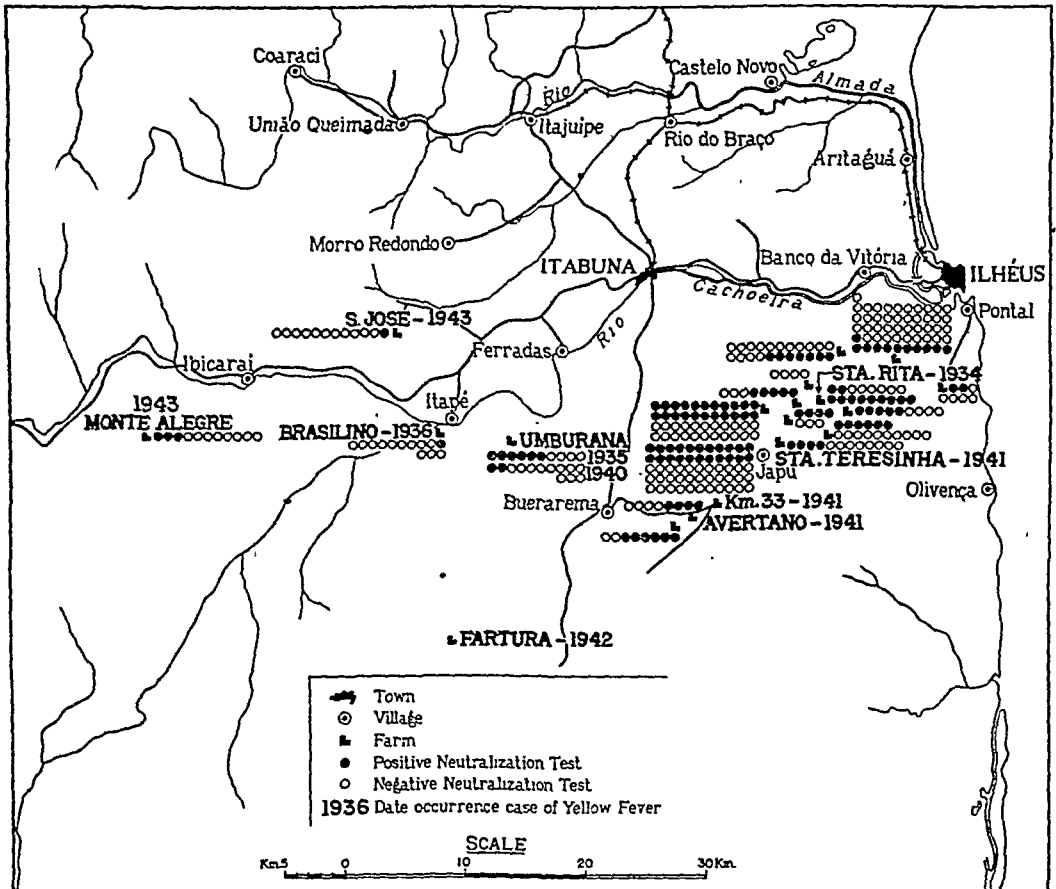


MAP 1. Location of Study Area

by *A. aegypti* could be definitely excluded. Blood samples were taken from the seven remaining members of the family; four were immune to yellow fever—one a boy of seven years of age. This may be considered as being the first proven case of yellow fever of jungle origin recognized in this region.

Subsequently liver specimens positive for yellow fever were obtained from persons who died on the following dates: December 1935, January 1936, April 1940, two in June and one in December 1941, July 1942, and January 1943. The

deceased lived in widely separated rural areas in the southern portion of the *municípios* of Ilhéus and Itabuna (map 2). Epidemiological studies, also carried out by Dr. Virgílio de Oliveira, established the jungle origin of infection on each instance. In the course of the investigations blood samples were collected from the surviving members of the families of the deceased and from other persons living in the neighborhoods. A total of 311 blood specimens were thus obtained and tested for the presence of antibodies neutralizing yellow fever virus. It was



MAP 2. Date of occurrence of yellow fever of jungle origin diagnosed from liver specimens and results of immunity surveys in the associated human population.

found that 37.3 per cent gave a positive neutralization test. The percentage of immunes (60.4) among persons above the age of 15 was much greater than among those below this age (18.6). The results of the immunological survey in relation to each death from yellow fever are also shown on map 2.

The periodic occurrence of yellow fever among rural inhabitants over a protracted period, combined with a high rate of immunity in the families and the neighbors of the deceased and the absence of *A. aegypti*, justified the conclusion that jungle yellow fever existed in this region in endemic form.

*Description of the Region:* The town of Ilhéus lies on the coast of the State of Bahia, 132 miles south of the city of Salvador and 114 miles north of Porto Seguro

where Cabral's galleons anchored in 1500. Its longitude is  $39^{\circ} 02'$  west and its latitude  $14^{\circ} 48'$  south (map 1). It has a small harbor formed by the estuary of the Cachoeira and Sant'Ana Rivers and is connected by a narrow gauge railway with the inland towns of Itabuna, Itajuípe and Urucúca (map 3).

The only road that is consistently suitable for automobile travel extends westward from Ilhéus and passes through Itabuna. This road, and the railroad that terminates at Itabuna, constitute the main arteries of mechanized transport and communication with the interior. Otherwise travel and transport of agricultural produce and merchandise is negotiated along streams in canoes or over unimproved dirt roads and trails, on foot or on mule back.

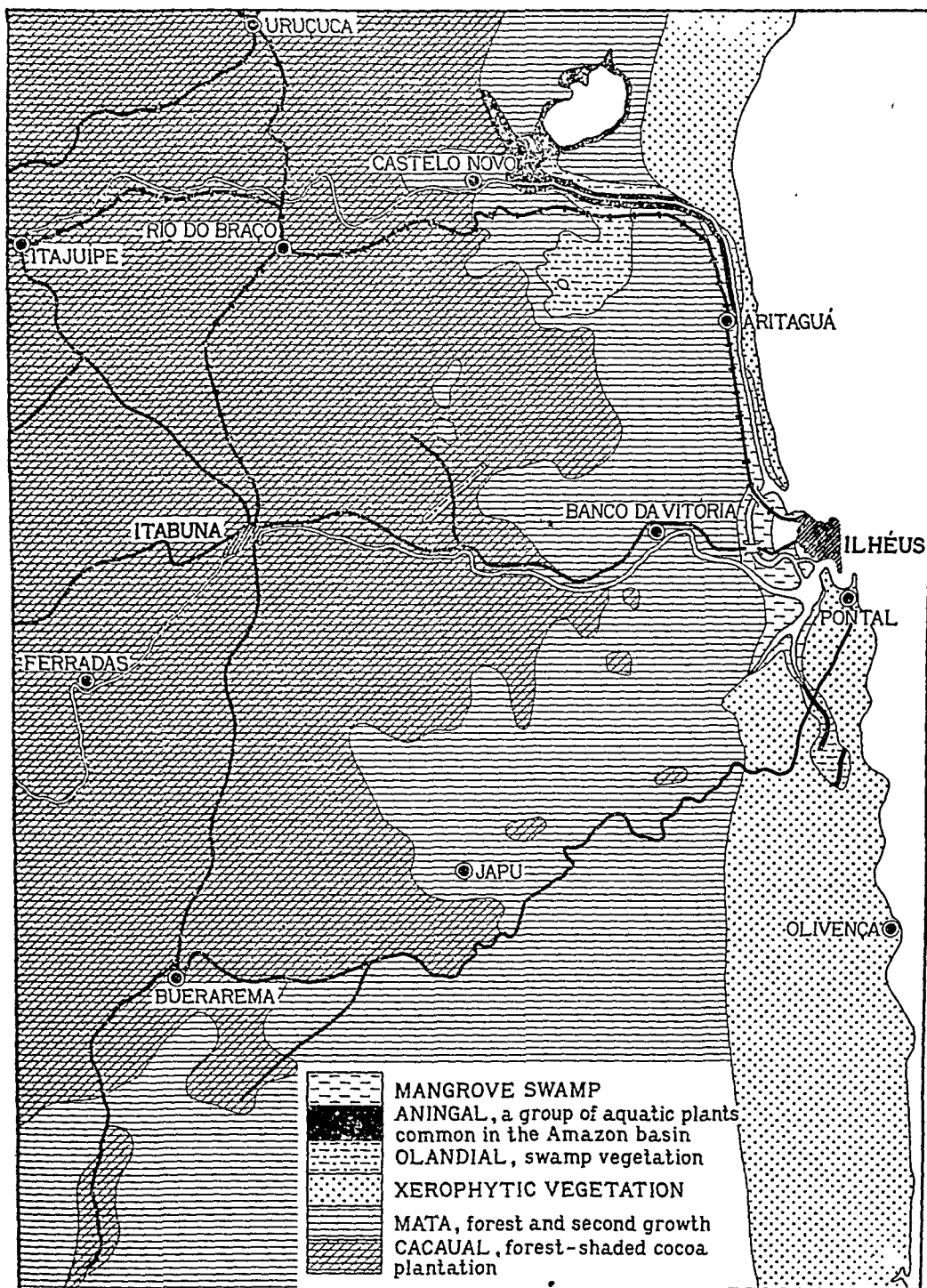
Bordering the sea is a flat strip of sandy soil varying in depth from a few hundred meters to several kilometers. This flat strip of sandy soil gives way to undulating hills, interlaced with small rapidly flowing rock-bed streams. As the hills are approached the soil changes from the sandy marine type to a dark reddish clay enriched by the decomposition of gneiss which appears in outcroppings on the sides of hills and the eroded banks of the streams. Geologically it belongs to the archaic formation of the Serra do Mar. Near the sea the hills are low and gently rolling, rarely exceeding a height of 100 meters, but further inland the terrain becomes more broken and the elevation gradually increases to join the central plateau.

The *climate* varies with the distance from the coast. Along the seaboard and inland for a depth of 50 kilometers or more, depending upon the altitude, it may be described as a humid tropical climate. The mean annual temperature is slightly above  $22^{\circ}\text{C}$ . and the monthly mean temperature never falls below  $18^{\circ}\text{C}$ ., nor does it vary more than  $6^{\circ}\text{C}$ . from month to month.

The *annual precipitation* usually exceeds 1,900 mm. and during the period (1924-1935) averaged 2,115.5 mm. The rainfall is fairly evenly distributed throughout the year but is commonly somewhat greater during the months of March, April and July, and again in November and December. A great part of the precipitation occurs in the form of thunder showers, and a day without some sunshine is infrequent. The heaviest and most equally dispersed rainfall occurs near the coast. Inland it tends to diminish and becomes more seasonal in character until the semiarid plateau region with its scanty and highly seasonal rainfall is reached.

The *predominant wind* from November to March is east by northeast, and during the remainder of the year it is east by southeast. The mean monthly wind velocity varies from 2 to 4 meters per second. It is usually somewhat stronger during the months of April, July and August. Except near the coast, where there is commonly a light to moderate breeze, there is little consistent air movement except preceding and during thunder storms, when it may for a limited period attain considerable velocity.

The character of the *vegetation* varies with the nature of the soil, rainfall, elevation and the distance from the coast. Bordering the sea and extending inland according to the width of the sandy soil, is a strip of halophilous vegetation among which planted coconut palms occur. Next comes the xerophilous zone which



MAP 3. Predominant type of vegetation in the *Study Area*

may be described as a transition between the halophilous and hygrophilous vegetation, and is characterized by the presence of cultivated piassava palms (*Attalea funifera*), scraggly forests or even patches of savanna. This zone, which is in-



definite in extent, blends into the massive hygrophilous humid to semihumid forests that commence a few kilometers from the coast and extend inland for 40 kilometers or more. The hygrophilous belt has two characteristics. The semihumid mesothermal vegetation which covers the tops of hills is peculiar to the Ilhéus region and can be clearly differentiated from the type which covers the tops of the Serra do Mar Mountains to the south. The humid megathermal vegetation covers the slopes of hills and lowlands and is essentially Amazonian in character; many of the same botanical species are found in both regions. At intervals along the coast, where the formation of sandbars or silt in the mouths of streams has obstructed the outflow of water, swamps covered with mangrove and other types of swamp vegetation have developed.

As the altitude and the distance from the coast increases and the rainfall diminishes and becomes more seasonal in distribution, the forests change from the humid and semihumid to the semiarid type. On the central plateau, where rainfall is scarce, the vegetation is xerophilous, consisting of stunted bushes, Cactaceae and Bromeliaceae. This general vegetational pattern extends from the Rio das Contas in the State of Bahia southward along the coast to the Rio Doce basin in the State of Espírito Santo.

The most characteristic feature, and the one which is of particular interest, is the broad band of humid forest with its dense upper canopy that lies between the coast and the elevated central plateau. This type of forest and the accompanying climatic conditions are encountered only here and in portions of the Amazon basin and, it would appear, constitutes an environment peculiarly adapted to harboring the virus of jungle yellow fever.<sup>3</sup>

The area chosen for more intensive study forms roughly a rectangle approximately 30 by 50 kilometers, bounded on the north by the Almada River which flows just south of Castelo Novo, on the west by Itabuna, on the south by Buarama, and on the east by the sea. This will be referred to subsequently as the *Study Area*. The more extensive and older forests extend along the east near the coast and to the south (map 3). In the strict definition of the term there remains only a very limited amount of virgin forest in the area. From most of the forests indicated on the map either selected timber has been cut or the land was at one time more or less completely cleared but has since returned to forest. There is also within this forested zone a rather extensive swamp lying between Aritaguá and Castelo Novo. The western portion of the area is largely devoted to the cultivation of cacao.

The zones, as shown on map 3, represent the predominant type of vegetation only. In reality there are small cultivated areas, principally of mandioca and some cacao, and patches of pasture land within the more extensive forested region. Likewise there are clumps of trees, usually second-growth, or partially lumbered forests on the hilltops and along the streams in the cacao zone, as well as some open grasslands for pasturing pack-mules and a few cattle. The cacao is grown

<sup>3</sup> A more detailed description of the flora of this region is to be found in the *Memorias do Instituto Oswaldo Cruz* (Rio de Janeiro), Vol. 44, Nos. 1 and 2, 1946, by Henrique P. Veloso.

under partial shade afforded by tall trees that spread their branches 5 to 10 meters above the dense foliage of the smaller cacao trees. Thus, there are two distinct canopies, the less homogenous one above, through which filters some sunshine, and the lower canopy formed by the cacao trees which virtually prevents all direct rays of the sun from reaching the ground. The floor of the old and well-developed groves is open and free of surface vegetation. These shaded plantations, with the interspersed patches of forest, constitute a not unfavorable habitat for certain species of arboreal animals, such as marmosets. There are two methods of establishing the cacao. The first and more common procedure is to clear the forest floor but to leave enough trees standing to furnish the required shade. The second method is to clear the forest completely and to plant the young trees in corn or mandioca which furnish temporary shade until the fast growing tree of the genus *Erythrina* that is planted at the same time attains sufficient height to give permanent coverage.

*Population:* The *municípios* of Ilhéus and Itabuna have a population of 208,000 or 26.7 persons per square kilometer. There are no exact figures on the proportion of people living in towns and villages and those living in isolated habitations. Although there are sparsely inhabited areas further inland and to the south, the population in these two *municípios* is relatively dense, considering that there is no manufacturing and that the people are dependent entirely upon agricultural pursuits. The *Study Area*, including the villages, has an estimated population of 31,000. Notwithstanding the rather extensive forests and a tendency to expand the land under cultivation, this is not a "frontier" region in the strict sense of the term, since Ilhéus has been established as a trading port for more than three centuries and cacao has been extensively produced for at least 75 years. Due, however, to the productiveness of the soil and the opening up of new territory further inland, there has been a considerable influx of labor during recent years from the less fertile northeast section of Brazil. This accounts for the excess (19.3 per cent) of males over females and a tendency of labor to shift from one location to another. In consequence, considerable difficulty was encountered in finding people who had been born and raised in the same locality.

*Economic and Living Conditions:* The principal export is cacao and the prosperity of the region fluctuates with its price on the world market. Other agricultural exports, consisting of coconuts, tapioca, kola nuts and fiber of the piassava palm are comparatively insignificant. Stimulated by war demands, a few rubber trees planted many years ago have been exploited and new plantations are being undertaken south of the *Study Area*. There is also limited shipment through Ilhéus of beef and hides from the interior.

Most of the cacao plantations are rather large and are conducted by hired labor. Thus, the rural population is sharply divided into two economic classes, the land owners or their estate managers, and the much more numerous common laborers or tenants. Our survey involved principally the latter class. With the exception of the houses of the estate owners or managers, the rural habitations are very primitive. The walls are constructed of mud held together by a network of slender poles. The roofs are usually made of palm leaves and the floors are of

dirt. Rarely are the mud walls surfaced either on the outside or inside. Frequently there are no windows and the door space may be left open or covered with reeds or sackcloth. On the estates of some of the more considerate owners, the tenant houses are better built and have tile or tin roofs, windows with glass panes, and doors. But in no instance are there any sanitary provisions, and the chickens, dogs and pigs, when possessed, have free access to the house. Screening is unheard of even in the houses of the estate owners, and insects of all kinds may enter at will. There is usually some cleared land about the house but the extent of the clearing is variable and some of the houses are in close proximity to cacao groves or forests.

The monocultural system reflects unfavorably upon the economic and nutritional status of the laboring population. Most of the cereals consumed in the region are imported. Mandioca is the only staple food produced in adequate quantity. Garden vegetables are scarce. Consequently, the standard diet consists principally of rice, black beans, mandioca, fruit, a minimum of meat, and some fish along the coast.

#### PLAN AND SCOPE OF STUDY

The study incorporated what may be termed "direct" and "indirect" methods. It was hoped that the evidence accumulated from the one would complement and support that assembled from the other.

Under the "direct" method may be classed effort to isolate the virus and to trace its passage through man and animals by a survey of the immunity which infection with the virus confers. These efforts consisted of: (a) search for the virus in mosquitoes and ectoparasites by inoculating suspensions of triturated arthropods into susceptible animals; (b) search for the virus in captured animals by inoculating their sera or suspensions of organs into other animals known to be susceptible, and (c) tests for the presence of specific neutralizing antibodies in human sera and sera of captured forest animals.

Under the "indirect" method may be grouped efforts to determine the habits and environment which tend to favor human infections, and the combination of flora and fauna conducive to the maintenance of the virus in forests. These comprised: (a) a survey of the flora of the region; (b) a survey of the animal population in different types of vegetation, and (c) a survey of mosquitoes, likewise in different types of vegetation. Finally, all of the accumulated data were analyzed with the object of determining whether there was any relation between the present or past existence of the virus, as revealed by the direct methods, and the habits of the people, their environment, or the floral and faunal characteristics of the forests, as revealed by the indirect methods.

For convenience of presentation and also because man probably plays no intrinsic part in the maintenance of the virus in the forests, the data are recorded in two parts. The first part deals with the epidemiology of human infections, and the second part with investigations on possible animal hosts and arthropod vectors of the virus.

## IMMUNITY SURVEY OF THE HUMAN POPULATION

*Collection of Blood Specimens:* Shortly preceding and during the early stages of the field study, blood samples were collected in "venules" of 8 to 15 cc. capacity from persons residing in the *município* of Ilhéus and in the neighboring *município* of Itabuna. The serum was separated from the blood clot, usually on the second or third day following collection, centrifuged if required, transferred to glass ampules, sealed and sent to the Rio de Janeiro Laboratory by air express for testing. In some instances, before the installation of the field laboratory at Pontal, the venules were sent direct to Rio de Janeiro and the serum was separated there. Little difficulty was encountered in the way of bacterial contamination.

The survey was confined to the rural inhabitants and, with few exceptions, was limited to persons born in either one of the two *municípios*. An effort was made to obtain blood samples from a relatively large number of children (below the age of 15 years) in order to determine the more recent presence of the virus.

Approximately 15 months later, near the close of the field study, a second sample of blood was taken from persons who had given a negative neutralization test at the first bleeding, in order to ascertain if infection with yellow fever virus had occurred during the interim.

*Collection of Epidemiological Data:* In addition to data on age, sex, place of birth, length of time in present residence, profession and economic status, information was obtained on the frequency of visits to old and young forests, and on excursions of more than 5 kilometers by train, autobus, horseback and on foot. On a second form, which served for all members of a household, the location and type of dwelling, its immediate surroundings and its distance from old and young forest was recorded. It was hoped that by the analysis of such data, some conception might be obtained of the personal habits and residential environment that favored infection.

No effort was made to secure history of past illness, since such information has been shown to be rather unreliable in identifying yellow fever infection, even following an acute epidemic (Soper and Andrade (13)). It is believed, therefore, that such information is valueless under endemic conditions.

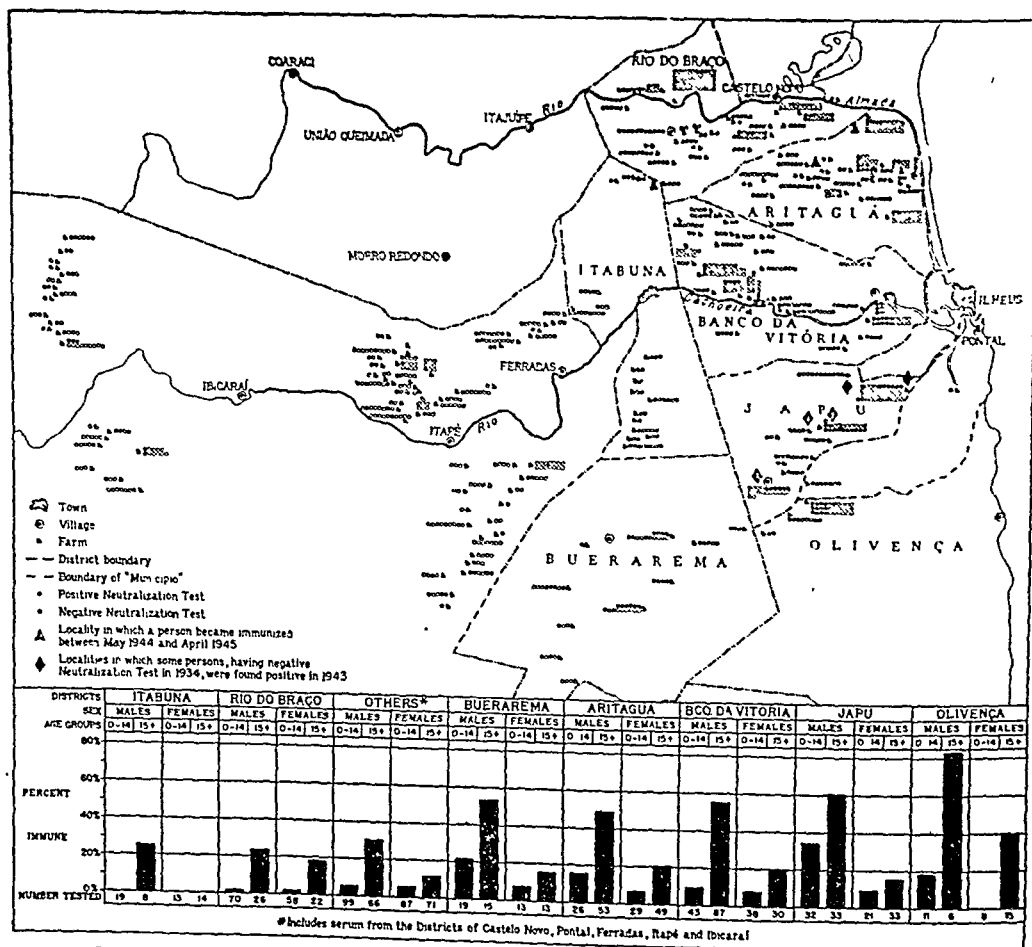
## LABORATORY METHODS

*Neutralization Test:* Neutralization tests were performed according to the method of Theiler (14), utilizing the technique of intracerebral injection. The virus used was the neurotropic French strain in its 520th serial mouse brain passage. One standard preparation of desiccated virus-containing mouse brain was used in all of the tests. This virus preparation was so diluted, in a 10 per cent concentration of normal monkey serum (*Macaca mulatta*) in 0.85 per cent NaCl solution, that approximately 200 LD<sub>50</sub> were contained in a volume of 0.03 ml. Quantities of 0.2 ml. of this virus dilution were mixed with an equal volume of the undiluted serum to be tested. This serum-virus mixture was allowed to stand for one hour at a temperature of 37°C., and was then injected intracerebrally into a group of six Swiss mice, each mouse receiving 0.03 ml. The mice were kept under observation for a period of 10 days. Two series of controls were used in each test: the first consisted of a titration of the virus content in tenfold dilutions and the second consisted of a titration of a standard immune serum in fourfold dilutions.

*Complement-Fixation Test:* The method used in the complement-fixation tests is essentially that described by Lennette and Perlowagora (15) with the exception of a modification of the preparation of antigen (16). In our tests a purified antigen, prepared by the extraction of the lipids and the separation of the globulin fraction of infected mouse brain, was substituted for the whole brain antigen previously used. This modification gave an increased specificity, and avoided a great majority of the non-specific fixations encountered when testing sera from syphilitic donors. Two units of complement were employed. The period of fixation consisted of one hour at 37°C., and overnight storage in the refrigerator. Only sera showing complete fixation in a minimum dilution of 1:8 were considered to be positive.

## RESULTS

**Geographical Distribution of Yellow Fever Immunity:** Persons giving immune reactions to yellow fever virus are found among rural inhabitants throughout the entire region surveyed, with the exception of those living north of the Rio Almada (map 4). The percentage of positives is greater in the districts of Banco da Vitória, Japu and Olivença, situated in the southern portion of the *município* of



MAP 4. Distribution of immunity to yellow fever in the human population

Ilhéus. In each of these districts the percentage of immunes is above 30. By comparison with map 3, it will be observed that the districts containing the higher percentage of immunes also contain or border upon the more extensive forests. The limited number of blood samples taken in the *município* of Itabuna also reflects a wide distribution of immunity, although the percentage of positives is lower (11.2) than in the southern portion of the *município* of Ilhéus.

**Relation of Age and Sex to Yellow Fever Immunity:** In general the incidence of immunity, as determined by the neutralization test, increases with age (table 1, fig. 1). Exceptions to this rule occur in the age groups of 0-4 in males and 40-49 in females, but in both instances the numbers involved are small and the dif-

ferences from the neighboring age groups are scarcely significant. It is not improbable that the higher percentage of positive reactors in the age group of 0-4, as compared with the age group 5-9, was occasioned by a conscious effort to obtain blood samples from young children in localities where the presence of yellow fever virus in the neighboring forest was suspected. The greatest difference in immunity occurs between the consolidated age groups of 0-14 and over 15 years of age. The percentage of immunes below the age of 15 is only 7.5, while among

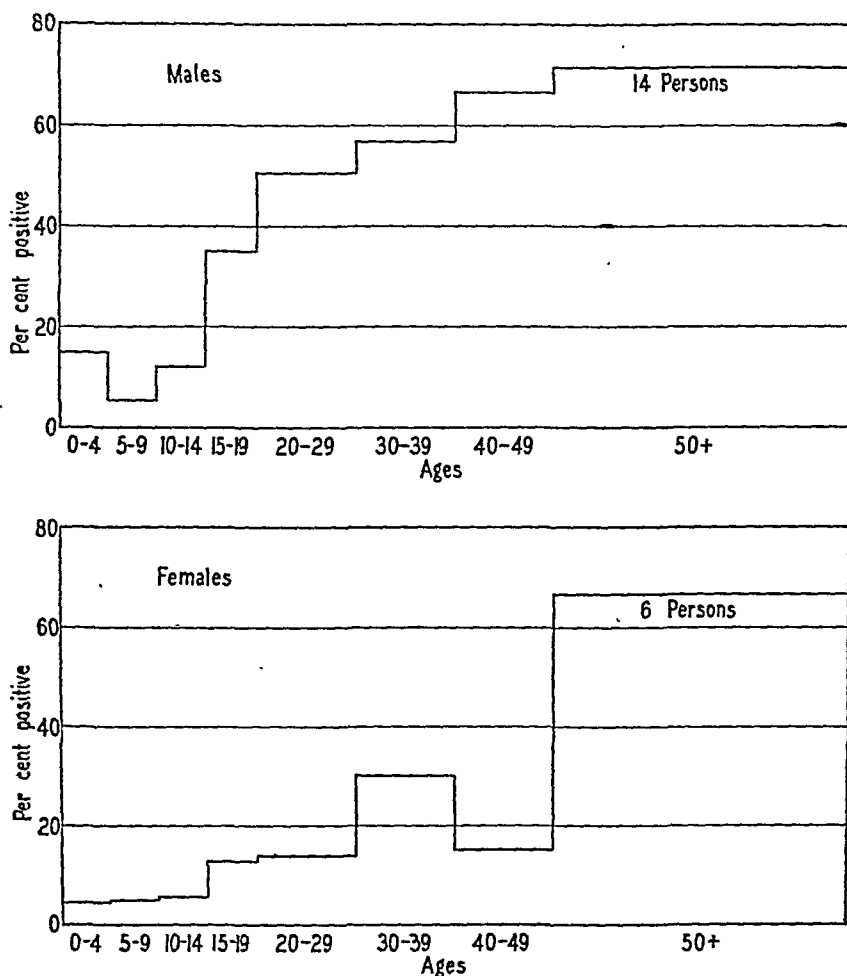


FIG. 1. Relation of Age and Sex to Yellow Fever Immunity

those 15 years of age and above the rate is 32.7, or 4.4 times greater. Although a rise in immunity with age may be expected in the presence of an endemic disease if exposure and susceptibility are more or less constant throughout life, apparently some factor existed which increased the probability of infection, especially among males, after the age of 15. Among males the ratio below 15 years of age is 9.5 but in the next 15 years (15-29 age group) it is 41.8 or 4.4 times greater.

The global immunity ratio of males is 2.6 times greater than of females. The difference is especially marked in the age group of 15 years and above. In the

older age group the immunes among the males are 2.8 times greater, while in the age group below 15 the immunes among the males are only 1.8 times more frequent than among females.

*Relation of Occupation to Yellow Fever Immunity:* In this, and in the succeeding analyses, are included only persons who were born in the *municipios* of Ilhéus or Itabuna, and who were residing within the *Study Area* at the time of the survey. This group comprises 707 individuals.

The highest percentage of immunes is encountered among the laborers and managers on cacao plantations, followed by woodcutters and those engaged in the cultivation of mandioca (table 2). The immunes among those occupied in household duties are significantly fewer, and as was to be expected, the children

TABLE 1  
*Relation of age and sex to yellow fever immunity*

AGE GROUPS	MALES			FEMALES			BOTH SEXES		
	Number Tested	Number Positive	Per cent Positive	Number Tested	Number Positive	Per cent Positive	Number Tested	Number Positive	Per cent Positive
0-4	27	4	14.8	24	1	4.2	51	5	9.8
5-9	143	8	5.6	103	5	4.9	246	13	5.3
10-14	158	19	12.0	157	9	5.7	315	28	8.9
0-14	328	31	9.5	284	15	5.3	612	46	7.5
15-19	134	47	35.1	104	13	12.5	238	60	25.2
20-29	103	52	50.5	101	14	13.9	204	66	32.4
30-39	44	25	56.8	30	9	30.0	74	34	45.9
40-49	15	10	66.7	20	3	15.0	35	13	37.1
50+	14	10	71.4	6	4	66.7	20	14	70.0
15+	310	144	46.5	261	43	16.5	571	187	32.7
Total all ages....	638	175	27.4	545	58	10.6	1,183	233	19.7

(under 15 years of age) listed under "no occupation" gave the lowest rate. Not only is there a higher ratio of males engaged in the more hazardous occupations, but the percentage of immunes among this sex is greater than in the females employed in the same type of work.

*Relation of Visits to Old-Type Forests to Yellow Fever Immunity:* Because there is considerable variation in forest aggregates within the *Study Area*, exact and detailed classification is rendered difficult since not infrequently one type is intermixed and blends in with another. Moreover, the limited number of persons involved in the survey does not permit division into a large variety of contact groups and still afford a significant number in each group. It was therefore decided, for purpose of contact analysis, to classify the forests into two categories according to age, one comprising forests 75 years or more of age, and the other younger forests, or forests less than 75 years of age. This division, though ar-

bitrary, seems logical as after about 75 years a forest in this region has largely reverted to the climactic or virgin type which differs in number and variety of

TABLE 2  
*Relation of occupation to yellow fever immunity*

OCCUPATION	MALES		FEMALES		BOTH SEXES	
	Number Tested	Per cent Positive	Number Tested	Per cent Positive	Number Tested	Per cent Positive
Laborers, cacao plantations.....	165	47.9	78	25.0	243	40.7
Managers, cacao plantations.....	8	37.5			8	37.5
Wood-cutters (charcoal).....	7	28.5			7	28.5
Laborers, mandioca farms.....	62	27.4	61	11.5	123	19.5
Domestic.....	2	50.0	67	10.4	69	11.6
No occupation (children).....	136	8.8	115	5.2	251	7.2
Miscellaneous.....	6				6	
Total.....	386	29.8	321	12.2	707	21.8

NOTE: This and the following tables include only persons living within the *study area* and who were born in the *municípios* of Ilhéus or Itabuna.

TABLE 3  
*Relation of visits to old-type forests to yellow fever immunity*

VISITS		MALES			FEMALES			BOTH SEXES		
		0-14	15+	Total	0-14	15+	Total	0-14	15+	Total
Daily	Tested	10	48	58	4	15	19	14	63	77
	Positive	6	32	38	0	7	7	6	39	45
	Per cent Positive	60.0	66.7	65.5		46.7	36.8	42.9	61.9	58.4
Weekly	Tested	34	35	69	15	26	41	49	61	110
	Positive	6	23	29	2	8	10	8	31	39
	Per cent Positive	17.6	65.7	42.0	13.3	30.8	24.4	16.3	50.8	35.5
Monthly	Tested	10	22	32	2	9	11	12	31	43
	Positive	3	8	11	0	2	2	3	10	13
	Per cent Positive	30.0	36.4	34.4		22.2	18.2	25.0	32.3	30.2
Never	Tested	150	77	227	146	104	250	296	181	477
	Positive	10	27	37	7	13	20	17	40	57
	Per cent Positive	6.7	35.1	16.3	4.8	12.5	8.0	5.7	22.1	11.9

botanical species from the younger immature forests. These categories will henceforth be designated as "old-type forests" and "young-type forests." The extent of the "swamp forests" and the "transition zone forests" and the human



contacts with these types of vegetation were too limited to warrant separate classification.

In the total population surveyed (all ages and both sexes) there is a definite relation between visits to old-type forests and immunity to yellow fever (table 3). It is especially manifest among the group making daily visits, and while the difference between weekly and monthly visits is not significant, those who make either weekly or monthly visits are much more prone to become infected than those who deny contact. The highest ratio of immunes occurs in the males

TABLE 4  
*Relation of visits to young-type forests to yellow fever immunity*

VISITS		MALES			FEMALES			BOTH SEXES		
		0-14	15+	Total	0-14	15+	Total	0-14	15+	Total
Daily	Tested	48	77	125	27	53	80	75	130	205
	Positive	7	39	46	4	16	20	11	55	66
	Per cent	14.6	50.6	36.8	14.8	30.2	25.0	14.7	42.3	32.2
	Positive									
Weekly	Tested	70	45	115	47	40	87	117	85	202
	Positive	6	21	27	3	9	12	9	30	39
	Per cent	8.6	46.7	23.5	6.4	22.5	13.8	7.7	35.3	19.3
	Positive									
Monthly	Tested	13	6	19	11	6	17	24	12	36
	Positive	1	2	3	1	3	4	2	5	7
	Per cent	7.7	33.3	15.8	9.1	50.0	23.5	8.3	41.7	19.4
	Positive									
Never	Tested	73	54	127	82	55	137	155	109	264
	Positive	11	28	39	1	2	3	12	30	42
	Per cent	15.1	51.9	30.7	1.2	3.6	2.2	7.7	27.5	15.9
	Positive									

15 years and over who visit forests frequently, and the lowest among those (both sexes) under 15 years of age who deny entering forests. The number in the latter age group that visits old-type forests is small but the ratio of immunity among the aggregate of those who make daily, weekly or monthly visits is significantly greater than among those who do not make such visits. The effects of visits are also manifest among the females of the older age groups although the immunity rate is not so high as among the males.

Visits to old-type forests are more commonly made by males (41.2 per cent) than by females (22.1 per cent), and by those 15 years of age and above (46.1 per cent) than by those in the lower age group (20.2 per cent).

The implications of these differences in age and sex immunity and the circumstances which may favor infection of men in preference to women will be considered in the "Discussion" (p. 19).

*Relation of Visits to Young-Type Forests to Yellow Fever Immunity:* The correlation of immunity with the frequency of visits to young-type forests is not so striking, although the global immunity rate among those who make daily visits is significantly higher than among those who make visits weekly or monthly (table 4). The rate of immunity in those making weekly or monthly visits, while slightly higher, does not differ significantly from that of the group which denied such visits.

*Relation of Visits to Old-Type Forests and Young-Type Forests to Yellow Fever Immunity:* A certain number of people gave a history of having visited both old-type forests and young-type forests, and in order to obtain a clearer conception of the effect upon immunity of visiting each type of forest, divisions were made into those who visited old-type forests only, young-type forests only, both and neither type of forest. The results are listed in the following tabulation:

Visits to:

	OLD-TYPE FOREST ONLY			YOUNG-TYPE FOREST ONLY			NEITHER	BOTH	TOTAL
	Daily	Weekly or monthly	Total	Daily	Weekly or monthly	Total			
Immunes.....	24	11	35	29	21	50	7	62	154
Total.....	35	35	70	118	165	283	194	160	707
Per cent immune..	68.6	31.4	50.0	24.6	12.7	17.7	3.61	38.8	21.8

It is apparent that persons visiting old-type forests are more frequently immune than those visiting young-type forests. Nor do visits to young-type forests in addition to visits to old-type forests appear to enhance the chance of acquiring immunity. However, those visiting young-type forests only, especially if the visits are made daily, are more likely to be immune than those who visit no forests whatever.

*Relation of Distance of Habitation from Old-Type Forests to Yellow Fever Immunity:* The number of people included in the survey who lived within 100 meters of old-type forests is small and, while the infection rate among them is somewhat greater than among those who lived at a distance of 101-500 meters, the difference is not statistically significant. However, the immunity rate is significantly higher among those who live within 500 meters of a forest than among those who live at a greater distance ( $14.2 \pm 2.6$  per cent).<sup>4</sup>

In assessing the significance of this relationship a second factor must be taken into consideration, namely, persons living in the vicinity of old-type forests tend to visit these forests more frequently than do those who live more than one-half kilometer distant. Further analysis shows that the ratio of immunes among those who live within 500 meters, but who do not visit the forest, is no higher than among persons who live at a greater distance and likewise do not visit such forests. It appears, therefore, that living in the vicinity of a forest, unless coupled with visits to the forest, bears little if any relation to immunity.

<sup>4</sup> P.E. or  $0.6745 \times \text{S.D.}$

*Relation of Distance of Habitation from Young-Type Forests to Yellow Fever Immunity:* While immunity is slightly higher among those living within 100 meters of young-type forests than among those living at a greater distance, the difference is not significant ( $6.8 \pm 3.2$  per cent). It may be inferred that living in proximity to young-type forest is not definitely associated with yellow fever immunity.

*Travel in Excess of Five Kilometers and its Relation to Yellow Fever Immunity:* Information on journeys in excess of 5 kilometers from the place of residence was elicited for the purpose of evaluating the possible influence of travel upon the immunity rate. Classification was made according to whether journeys were made weekly, monthly, yearly, or never; and whether by train, autobus, horseback or on foot. It was found that travel by the first three means was very insignificant. Only 26.7 per cent of the people admitted to travel by train, 16.5 per cent by autobus and 6.5 per cent by horseback. However, 52.3 per cent made trips greater than 5 kilometers on foot and 42 per cent were accustomed to make such trips weekly or monthly. Most of the persons included in the survey belong to the lower economic status which no doubt accounts for the infrequency of travel by train or autobus. Few of them own horses or mules, and even if they do the animals are used for carrying cargo and not for riding.

There was found to be no relation between yellow fever immunity and travel by train, autobus or horseback, but there is evidence of positive correlation between frequency of travel by foot and immunity. Again other factors must be considered in interpreting this correlation. The persons who travel most frequently are males of 15 years of age and above. It has been noted that the highest percentage of immunity occurs in this sex and age group. In addition, those who travel by foot are also given to visiting old-type forests. Correction for these factors leaves considerable doubt as to whether travel *per se* is conducive to yellow fever infection.

*Complement-Fixation Test:* The complement-fixation test was satisfactorily performed on 490 sera, 185 of which gave a positive and 305 a negative neutralization test. Of the 185 which gave a positive neutralization test 118 (63.8 per cent) also gave a positive complement-fixation reaction. Of the 305 negative neutralization test sera, two (0.7 per cent) were positive for complement fixation.

While there is a correlation between the neutralization and complement-fixation tests, it is not complete. The fact that only 63.8 per cent of the sera which neutralized the virus were also capable of fixing complement in the presence of the virus antigen may be explained by a tendency of the complement-fixing antibodies to disappear, in contrast to the permanency of the neutralizing antibodies. Thus, the complement-fixation reaction in combination with the neutralization test may be of value in estimating the time when the infection occurred. As yet, however, we do not have adequate data to estimate the length of time the complement-fixing antibodies persist after infection. In this instance the persons showing positive complement-fixation reaction were well scattered over the area and gave no indication of recently occurring infections in any given locality.

The two sera that fixed complement but failed to neutralize the virus, probably

represent non-specific reactions which occasionally occur even when purified antigen is used.

#### RE-SURVEY

Approximately 15 months after the original survey was made, blood specimens were taken from 234 persons who had given a negative neutralization test on the first survey. Only one of these, an adult male, was positive on the second survey. The specimen was taken at the close of the study period and it was not possible to secure information on his movements after the time the first blood sample was taken.

The second survey necessarily comprised people who had given a negative reaction on the first survey and of whom a rather high proportion were females and in the age group below 14 years. It will be recalled that this sex and age group is the least exposed to infection. Had there been more adult males included in the second survey, it is possible that more positives would have been found.

During the first survey made at the beginning of the study, blood specimens were obtained from a few persons who had been tested in 1934 in connection with the first yellow fever positive liver received, that of a child dying at Sta. Rita. A group of 26 persons living in the vicinity of Sta. Rita and Engenho Sant'Ana, who gave negative reactions in 1934, were retested and six of them were found to be positive approximately 10 years later.

#### DISCUSSION

It will be convenient at this point to discuss some of the data related to the epidemiology of the disease in humans before proceeding to the studies on animals and arthropods.

The present survey of the human population, besides being a corollary to the animal and arthropod investigations, was undertaken with the hope of gaining more precise knowledge of the factors influencing infection by a comparison of the living environment and personal habits of those who had been infected and those who escaped infection.

In the neutralization test we possess a rather highly reliable means of determining whether a person has been previously infected. There is ample evidence that human infection is followed by staunch immunity, manifested by the persistent presence of circulating antibodies capable of neutralizing the virus. It may be assumed, therefore, that a person possessing neutralizing antibodies has been infected with the virus at some prior occasion. Hence, the term immunity, as used here, implies previous infection.

The unequivocal association of immunity with visits to forests, especially the old type of forest, is the outstanding feature revealed in these studies. Indeed, it is believed that all other correlations with immunity, such as occupation, living environment and travel on foot, as well as the peculiarities of age and sex distribution, may be accounted for by contact with forests. In other words, personal contact with forests appears to be the dominant factor which

favors infection and the other correlations are partial and exist only because they in turn are related to visits to forests.

The observed age and sex distribution of immunity with its significantly higher ratio among males above 15 years of age is characteristic of the jungle form of the disease and is in sharp contrast to that found following urban epidemics of yellow fever transmitted by *A. aegypti* (6, 10). In the latter, women and children, and persons who spend most of their time in or near the house, are equally, if not more frequently, infected than the adult or adolescent male. The selection of males above the age of 15 years in the jungle form of the disease is in all probability attributable to their occupation and personal habits that lead to more frequent and more prolonged contact with forests and, consequently, with vectors of the virus, rather than to differences in age or sex susceptibility. The lower rate of infection among women engaged in the same occupation, who claimed to have visited forests as frequently as men, may be due to a difference in the quality of the work they perform and the nature and length of their visits to the forest. Both on the cacao plantations and in forests, women do not as a rule perform the same kind of work as the men and their visits are likely to be of shorter duration. They rarely engage in cutting of wood and clearing of underbrush. Also, it has been observed that male laborers while at work may wear only sleeveless undershirts, or are even stripped to the waist, and thus would be more exposed to the bites of insects. These differences, though not subject to statistical expression, may be important nonetheless.

The occupations which correlate with immunity are out-of-doors and are also likely to lead to forest contacts. The people who engage in such occupations are the ones most likely to enter or work alongside a forest.

While there is a higher rate of immunity among inhabitants of districts that incorporate, or border upon, extensive forests, an analysis of the more intimate proximity of human habitations to forests and immunity does not reveal any definite relationship when correction is made for the influence of visits to forests, i.e., people who live close to forests (less than 500 meters) also tend to enter the forest more frequently than those living at greater distances and when allowance is made for this factor, the close proximity of the habitation to forests loses its significance. It may be inferred, therefore, that the vectors of the virus do not ordinarily enter or remain in the immediate vicinity of houses. If this were true one would expect to encounter a high rate of immunity among women and children, and among those who remain at home. It cannot be said, however, that infection may not occasionally occur in the vicinity or even within the house, as the known vectors of the virus have been captured, though rarely, indoors. But in comparison with entering a forest the hazard of becoming infected in or about the house is relatively slight.

Finally, explanation for the correlation of immunity with travel by foot for distances of 5 kilometers or more may also be found in contact with forests, since the same people also frequently enter forests. Thus, it may be said that those who are given to infection tend to travel, but it is not to be implied that travel in itself favors infection.

Previous epidemiological investigations on jungle yellow fever in Brazil have been made in zones where the disease was transient or, according to the applied definition, epidemic. The human studies, which were directed largely to the investigation of fatal or clinically manifest infections, revealed that almost invariably the infected person had been in contact with forests within five or six days preceding onset of symptoms. The only group immunity survey furnishing classified statistical information on the relation of immunity to place of habitation was made by Burke (3) who found that the immunity ratio among persons living in an urban center was much lower than among the neighboring rural inhabitants, and that in the latter group the highest rate was encountered among those living within 3 kilometers of "fields and jungle." The present study, made in a region where the disease is endemic, confirms the earlier observations that contact with forest is the salient feature associated with infection. But it does not substantiate the inference that the location of the habitation in reference to the forest is in itself important. The disease affects rural inhabitants living near forests because these people are more likely than others to enter the forest. It may also be concluded that it is an occupational disease only in so far as the occupation leads to forest contact.

The extent to which these observations harmonize with the studies on forest hosts and vectors can be more appropriately discussed following the presentation of data in Part II.



## PART II. INVESTIGATION OF VERTEBRATE HOSTS AND ARTHROPOD VECTORS

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### REVIEW OF LITERATURE

During the years following the first laboratory passage in 1928 of yellow fever virus to monkeys, a mass of information has been accumulated on the susceptibility of vertebrates to infection with the virus and the ability of arthropods to retain or transmit it. A classified list of publications on this subject will be found in the August 1945 issue of the Tropical Diseases Bulletin. In the following review reference will be made only to articles of pertinent interest. We shall, however, refer to some unpublished data.

In interpreting the practical implications of the experimental work on vertebrate hosts and arthropod vectors of the virus, consideration must be given to the methods and criteria adopted in determining the susceptibility of a vertebrate and the ability of an arthropod to transmit the virus. Also the knowledge gained in the laboratory should be weighed in the light of field experience and the ecology of the host and vector involved. The one may serve to complement or to contradict the other.

As may be expected, the behavior of the virus and the response of the host are influenced by the dose of the virus employed and the route and manner by which it is introduced. It is fair to assume from the evidence at hand that natural infection of animals probably results from introduction of the virus through the epidermis by the bite of an infected insect. If this be true, the experimental infection of animals by the intracerebral, intraperitoneal, or intramuscular route has no counterpart in nature and tests for susceptibility based upon such techniques may be questioned as far as the epidemiology of the disease is concerned. On the basis of laboratory experiments only animals that may be infected by inoculation of the virus intradermally or subcutaneously, or preferably by the bite of an infected insect, should be regarded as potential hosts. It should also be borne in mind that for an animal to play a role in cyclic transmission of the disease the virus, following introduction, must multiply to such an extent as to attain a concentration in the blood sufficient to infect the insect vectors. It has been found, for example, that some species of mammals following exposure to infected mosquitoes may circulate virus in low concentration and subsequently develop neutralizing antibodies and yet be unable, or only rarely able, to pass the infection on to normal mosquitoes. It is not improbable that such low-grade infections giving rise to circulating antibodies may occur in nature when mosquitoes carrying the virus are present. Distinction should be made, therefore, between these low-grade, or so-called "dead end" infections, and those in which the virus circulates in adequate concentration to be readily transmissible to an insect vector, since the former can play no part in the continuous propagation of the virus in nature. It is obvious that the best laboratory



means of ascertaining whether an animal may be integrally involved in the epidemiology of the disease is to determine if the virus may be maintained for a series of cycles by alternate passage through the animal and a known efficient vector. Indeed, in its epidemiological application it would be desirable to limit the term susceptibility to vertebrates that satisfy this criterion.

Comparison and evaluation of reported laboratory experiments are further complicated by differences in host affinity among some of the jungle strains of the virus. It appears to be fairly well established that whereas certain mammalian species are rather easily infected by one strain of jungle virus, they are resistant to infection with another strain. Lastly, it has been observed that the neutralization test, while highly specific in man, and with few exceptions among the primates, is not so dependable in other mammalian orders (17). Apparently some of the marsupials, and especially certain species of rodents, possess non-specific virucidal substances in the blood capable of inactivating yellow fever as well as other neurotropic viruses (18). This phenomenon, which will be referred to later, tends to vitiate the results of immunity surveys in these mammals. The above remarks will serve to indicate that laboratory experiments on suspected hosts should be viewed critically and their epidemiological implications interpreted with caution.

*South American Vertebrates:* In experiments on South American vertebrates syringe inoculations of graded doses of the virus have been most frequently employed. "Susceptibility" was gauged by the presence or quantity of circulating virus, and the subsequent development of specific immunity. In some instances, confined principally to primates, infection was accomplished through the bite of infected mosquitoes. Recently cyclic passages in combination with mosquito vectors have been used to determine the ability of animals to serve as temporary hosts of the virus in these cyclic passages. These studies combined are quite extensive and cover a wide variety of vertebrates. They have yielded useful information and permit making three rough classifications of vertebrates according to their susceptibility.

1. *Those that are definitely susceptible.* Following dermal or subdermal introduction of the virus it rapidly multiplies and circulates for a period of several days, usually in high concentration, followed by demonstrable staunch immunity in the surviving host. Also, among those tested it has been found possible to maintain the virus in continuous cycles by alternate passage through an insect vector. This group probably embraces all of the South American primates. At least all that have been studied, including a rather wide range of species in both families of the order, can be infected by subcutaneous inoculation of the virus and all of those tested can likewise be infected by the bite of mosquitoes harboring the virus (19, 20, 21, 22, 23, 24, 25). The only exception was encountered by Bates and Roca-Garcia (26) who found difficulty in infecting a Colombian species of *Cebus* monkey, yet species of the Brazilian *Cebus* are quite readily infected with certain Brazilian strains of jungle yellow fever virus (22, 29). More recently continuous cyclic passages in combination with mosquito vectors have been reported in *Saimiri* (27), *Aotus* (28), *Cal-*

*lithrix aurita*, *Cebus versutus* (29), *Callithrix penicillata* and *Leontocebus chrysomelas* (30).

In addition, specifically immune monkeys have been repeatedly captured in forested areas where yellow fever is endemic and following epidemic excursions of the disease (3, 10, 26, 31). Finally, during the course of the studies here reported, yellow fever virus was isolated on four separate occasions from captured marmosets (32). Thus, the evidence against the primates in general, and in some species in particular, is very convincing. Bates and Roca-Garcia (26) have given a very good summary and discussion of the incriminating information concerning primates as well as marsupials.

2. *Those that are definitely not susceptible.* This includes all of the poikilothermal vertebrates examined (33). Also it is believed there may be placed in this group several of the bird families and all of the mammals belonging to the orders of Carnivora, Chiroptera, and Artiodactyla and probably most, if not all, of the Rodentia.

Although not exhaustively studied, the Chiroptera are placed in this group because the specimens that have been tested, representing fruit and insect eaters and vampire bats, were found to be decidedly resistant to infection (34, 35).

There have been published only a few observations on South American Carnivora (36, 37). They appear to be quite resistant and have been infected only when the virus was introduced intracerebrally. Laemmert has conducted some miscellaneous experiments (unpublished) with this order of mammals and none were found to circulate virus. The only exception to the generally negative results obtained with Carnivora is reported by Monteiro (38), who claims to have recovered virus from the blood of a dog three days, and from a cat 12 days, following inoculation.

Likewise the Artiodactyla have not been extensively studied, primarily because they are rather scarce in Brazilian forests in which jungle yellow fever has been identified. In Colombia, where peccaries occur in considerable numbers, four specimens of the species *Tagassu tajacu* were tested and two circulated small amounts of virus. Also one deer, *Mazama sp.*, was tested but failed to circulate virus (39).

3. *Those about which knowledge is incomplete or inconclusive.* There may be included in this group some, if not all, of the Marsupialia, Edentata, perhaps a few of the Rodentia and certain families of sylvan birds that have not been adequately studied. The interpretation of the laboratory investigations on this group is equivocal in that the virus may undergo multiplication and circulate in small to moderate amounts. Also, specific immunity may subsequently develop. It is uncertain, however, whether the amount of circulating virus is adequate for them to serve as hosts in cyclic transmissions under natural conditions. The interpretation of immune reactions in certain members of this group is confusing since their sera may manifest non-specific virucidal activity against yellow fever, as well as other neurotropic viruses (18).

The marsupials have been most extensively studied but the results have not always been consistent and in some instances have been contradictory. This

applies particularly to the relatively resistant members of the order. For example, Bugher *et al* (40) were able to demonstrate circulating virus in six of 56 (11 per cent) of *Didelphis marsupialis* following subcutaneous inoculation of a small dose of virus, while Laemmert (41) was unable to infect this species even with much larger doses of Asibi and Brazilian strains of jungle yellow fever virus. Using the same Colombian strain (Martinez) employed by Bugher *et al*, he was able to demonstrate circulating virus in only one of 23 animals of this species. However, Bugher *et al* (31) on the basis of positive neutralization tests in *D. marsupialis*, captured in forests where the virus was known to be present, deduced that this species was involved in the cyclic transmission of the virus. Yet the difficulty of consistently infecting this animal in the laboratory, the apparently low concentration of the virus in those infected, and the occasional presence of non-specific virucidal substances in the blood serum which may be confused with specific immunity to yellow fever, combine to cast considerable doubt upon the validity of this deduction. Laemmert was also unable (41) to infect *D. paraguayensis*. It would seem, therefore, that the members of the genus *Didelphis* are rather strongly resistant to infection.

Of the *Metachiroptops opossum*, while perhaps less resistant than individuals of the genus *Didelphis*, only a small per cent circulate virus following subcutaneous inoculation (39, 40, 41). According to the combined experiments of Bugher *et al* (40) and Bates (42) virus was recovered in four of 43 animals, and Laemmert (41) obtained virus from nine of 34 specimens inoculated. Bates was unable to syringe-pass the infection serially in this species (42).

*Caluromys philander* and *C. laniger*, woolly opossums, are certainly more easily infected than individuals of the foregoing genera (41), but there is not enough information on the amount of virus that circulates following infection to form an opinion on whether these species can serve as the vertebrate host in cyclic transmissions. In a few experiments Laemmert found that *C. philander* circulated virus following subcutaneous inoculation with two of the three jungle strains of virus employed. Bates and Roca-Garcia (26) succeeded in infecting *C. laniger* by the bite of mosquitoes in one of four trials. No continuous cyclic transmissions have been attempted.

The most complete studies have been made with *Metachirus nudicaudatus*. Both Bugher *et al* (40) and Laemmert (41) were able to infect *Metachirus* by subcutaneous inoculations and Bates (42) succeeded in making 10 serial passages by inoculating the serum from an infected animal intramuscularly into the next normal animal in the series. Later Bates and Roca-Garcia (26) found it possible to infect this species with mosquito vectors but were not successful, except occasionally, in transmitting the infection back to mosquitoes. However, Waddell and Taylor (43) have shown that the virus may be maintained in cyclic passages with *A. aegypti* and *Metachirus* by substituting a marmoset in every other host passage. Moreover some jungle strains, referred to later, may be maintained in cyclic passage with mosquitoes without the substitution of marmosets in the cycles.

The genus *Marmosa* includes a number of species. They are small fragile

animals, ill adapted to captive life. For this reason laboratory experiments are not very complete as they are frequently interrupted by premature deaths of the animals from extraneous causes. It is, therefore, difficult to carry through experiments on cyclic transmissions which demand a continuous supply of the host. They appear to be easily infected, perhaps with as great facility as the *Metachirus* (41), and Waddell and Taylor (43) have succeeded in carrying a jungle strain of virus through three *Marmosa cinerea*-*A. aegypti* cycles. The passages were discontinued for lack of additional marmosa.

The published studies on rodents have been confined almost exclusively to infection by intracerebral inoculation. This vicarious route of inoculation has no epidemiological significance and it is believed may be disregarded as far as susceptibility in the epidemiological sense is concerned. It may be mentioned, however, that a number of wild rodents have been infected by this method (36, 44, 45), and Bates and Weir (46) succeeded in making brain passages of yellow fever virus in a Colombian cane rat (*Zygodontomys*). Laemmert (47), testing many species of wild-caught rodents, has shown that several species circulate virus following subcutaneous inoculation. Some of these, such as the paca (*Cuniculus paca*), consistently circulate virus and develop a specific immunity. Other species, such as the squirrel (*Guerlinguetus ingrami*) and one species of *Oryzomys* and *Proechimys* respectively, also circulate small amounts of virus, but in an irregular manner, that is, some individuals of the species circulate virus and others do not. However, it has not been determined if the concentration of virus in the blood stream of these species is sufficient to infect mosquitoes. Recently Hughes (48) has shown that a species of *Proechimys* developed specific immunity following exposure to infected mosquitoes but only traces of virus circulated and it is highly improbable that these animals are capable of passing the virus to insect vectors. Species of *Oryzomys* and *Akodon* gave negative results when similarly tested. Even more than marsupials, certain rodents possess virucidal substances in their sera which may be confused with specific immunity. Consequently, positive neutralization tests in captured specimens usually do not indicate that the animals have been infected with yellow fever virus.

Some species of Edentates, notably armadillos (36, 39), have been infected in the laboratory. But again it is questionable if the virus circulates in adequate concentration to infect insect vectors. At least there is no proof that the infection may be transmitted in this manner. Moreover, the habits of the armadillos argue against their being concerned in what is known of the epidemiology of the disease.

Linhares (49) and Laemmert and Moussatché (50) have demonstrated that laboratory strains of virus, particularly strains manifesting neurotropism, may be passaged through the brains of young chicks but the latter (50) were not successful in making passages by the extraneural route, although circulating virus can usually be demonstrated for several days following inoculation. Very little has been published on forest birds and for this reason they have been placed in the group about which information is incomplete. However, L. C. Ferreira has conducted a considerable number of experiments at the laboratory

in Rio de Janeiro on the susceptibility of adult birds involving members of the following families:

<i>Tinamidae</i>	<i>Rallidae</i>	<i>Strigidae</i>
<i>Anatidae</i>	<i>Laridae</i>	<i>Coccyidae</i>
<i>Cathartidae</i>	<i>Columbidae</i>	<i>Thraupidae</i>
<i>Falconidae</i>	<i>Caculidae</i>	<i>Icteridae</i>
<i>Cracidae</i>	<i>Psittacidae</i>	<i>Ploceidae</i>
	<i>Fringillidae</i>	

It was inferred from these experiments that birds of the above-mentioned families are resistant to infection by the extraneural route of inoculation. Although a great many sylvan species of birds remain to be investigated, the laboratory evidence so far accumulated is essentially negative in character. Nor have immunity tests on captured specimens produced any definitely incriminating evidence.

In sum, it may be concluded that according to laboratory experience the only animals that comply with the proposed criterion for susceptibility in the epidemiological application of the term are primates and perhaps some of the marsupials such as *M. nudicaudatus* and, though information is incomplete, certain species of *Marmosa* and *Caluromys*.

*South American Arthropods:* Investigations on vectors of the virus have involved laboratory experiments on the ability of arthropods to retain the virus for varying periods following infection; on their ability to transfer the infection by bite to a susceptible vertebrate host; and on efforts to identify the virus in wild-caught specimens. It is quite important to distinguish between mere retention of the virus in the body of the arthropod and the capacity both to retain and to transmit the virus by bite, as it is the latter function which characterizes a vector of the disease as we now understand it.

In that yellow fever virus is capable of penetrating the epidermis, it is of course conceivable that the disease could be transmitted by the excreta of an infected arthropod being deposited upon the skin or by crushing of the body of the arthropod while it is feeding, as is known to occur in some of the rickettsial diseases. However, as will be discussed later, there is no epidemiological evidence supporting this manner of transmission among any of the vertebrates known to be susceptible to yellow fever virus.

A predominance of the work on arthropod vectors has been confined to mosquitoes although some miscellaneous experiments have been made with ticks, mites, fleas, lice, triatomas and haematophagous flies.

Aragão (51) reported transmission of yellow fever from an infected rhesus<sup>5</sup> monkey to normal monkeys of the same species by the bite of *Amblyomma cajennense* as well as by *Ornithodoros rostratus* that were allowed to feed upon a normal monkey three or four days following the infective meal. He also found that the virus survived in the body of the ticks for at least two weeks and

<sup>5</sup> Herein and henceforth in this paper the term "rhesus" will be used in place of *Macaca mulatta*.

in a single experiment was able to recover virus from the eggs of an infected tick. The eggs were deposited between the 11th and 14th day following the infection of the female and 11 days later the eggs were triturated and injected into a monkey. Davis (52) found that the virus may be retained by *Argas persicus* six days; *A. cajennense* 15 days; *Rhipicephalus sanguineus* 23 days; and *Boophilus microplus* (= *Boophilus annulatus microplus*) 10 days after feeding on an infected rhesus monkey. But he was unable to confirm the work of Aragão on transmission by bite or the passage of the virus from one generation of tick to another by egg.

Monteiro (53) claims to have demonstrated yellow fever virus in the feces of bed bugs (species not identified) collected between the 2nd and 12th day following feeding upon an infected rhesus monkey. Philip (54) was unable to transfer infection either by feeding *Cimex lectularius* or by inoculation of body suspensions following a "period of incubation." Using *Cimex hemipterus*, Kumm and Frobisher (55) were not successful in verifying the observation of Monteiro and found that the virus did not remain in the bodies of the bed bugs longer than two days following the infective meal.

It has been shown (56, 57) that the virus may be retained by adult *Triatoma megista* for eight to 10 days but not as long as 14 days. Out of six attempts one was successful in transmitting the virus by interrupted feeding, i.e., by permitting the insects to feed partially on an infected monkey and then transferring them to a normal monkey for completion of the blood meal, but after a period of incubation between meals no transmission occurred. Nymphs, following repeated feedings on infected monkeys, failed to show virus when tested 14 days after the last infective meal.

Philip (54) was able to transmit the infection by removing "100 or more *Pedicinus* sp., the common monkey louse" from an infected monkey and injecting a suspension prepared from the lice into a normal monkey. There is no information on the length of time the virus may be retained by the lice.

Davis (52) mentions in his paper on ticks that virus was demonstrable in chicken mites (genus or species not determined) six days following feeding on an infected monkey.

Hoskins (58) was unable to transmit the infection by interrupted feeding of dog fleas (*Ctenocephalides canis*) nor was the virus demonstrable in the bodies of the fleas 18 hours after the infective blood meal.

The same author (58) also conducted some experiments with *Stomoxys calcitrans*, and while this fly did transmit the infection by bite as long as six hours after the infective meal, it was not capable of doing so after 16 hours, nor did the virus remain viable in the bodies of the flies longer than 42 hours.

A considerable number of ectoparasites and species of *Simulium* and *Phlebotomus* have been collected in forests where yellow fever virus was known to exist and examined for the presence of the virus, with entirely negative results (31, 59).

The study of mosquitoes, as carriers of the virus both in nature and in the laboratory, has been much more exhaustive. Indeed virtually all of the genera,

if not all of the species, of mosquitoes found in significant numbers in association with yellow fever in South American forests have been subjected to investigation in one way or another. It will facilitate presentation of the evidence on mosquitoes to group them under the following headings:

*Found infected in nature:* This group includes the species of mosquitoes found to be harboring the virus when captured. It may be subdivided into (a) those in which the virus was demonstrated by permitting the mosquito to feed upon a susceptible animal (monkey) and (b) those in which the virus was identified by inoculating a suspension of triturated mosquitoes subcutaneously into monkeys, or intracerebrally into mice.

(a) Transmitted by bite: *Aedes leucocelaenus* (60), *Haemagogus capricornii*<sup>6</sup> (31, 59, 60), and possibly *Haemagogus lucifer*, and/or *Haemagogus equinus* (59).

(b) Virus identified by inoculation only: *Sabethini* spp. (60).

*Infected in the laboratory; transmitted by bite:* *Aedes scapularis* (61, 64), *Aedes fluviatilis* (56, 64), *Haemagogus janthinomys* (65) (probably *H. spegazzinii* (66)), *Haemagogus equinus* (29, 67), *Haemagogus spegazzinii* (29), *Haemagogus capricornii* (27) (see footnote<sup>6</sup>—probably *H. spegazzinii* subspecies *jalcó*), *Aedes leucocelaenus* (68), *Trichoprosopon frontosus* (69), *Aedes aegypti*. In addition there are three species concerning which the evidence is equivocal, or could not be consistently verified: *Aedes taeniorhynchus* (56, 64), *Culex fatigans* (70), *Psorophora ferox* (64).

*Infected in the laboratory; retained virus but failed to transmit it by bite:* *Aedes serratus* (61, 71), *Aedes terreus* (64, 71), *Aedes nubilis* (64), *Mansonia albicosta* (64, 70), *Mansonia fasciolata* (64, 71), *Mansonia chrysonotum* (64, 71), *Mansonia titillans* (55), *Mansonia justamansonia* (64), *Psorophora cingulata* (71).

From the epidemiological aspect, the finding of virus in wild-caught mosquitoes is of greatest interest. It has been isolated on 17 occasions from the genus *Haemagogus*, three times from *Aedes leucocelaenus* and once from *Sabethini*. When virus isolations were attempted all captured mosquitoes, irrespective of species were examined, so in this respect comparisons are legitimate. While the number of isolations is small it may reflect to some extent the relative importance of *Haemagogus* spp. and *A. leucocelaenus* as natural vectors of the disease. It will also be noted that both of these mosquitoes have been shown to be effective vectors in laboratory experiments. The unique isolation of virus from *Sabethini* was accomplished by inoculating intracerebrally into mice a suspension of a mixed group of 88 specimens composed of *Sabethoides*, *Limatus*, *Wyeomyia*, *Goeldia*, and *Trichoprosopon*. Twenty-one mosquitoes of the same group which were allowed to feed upon a normal monkey failed to cause infection.

<sup>6</sup> The names and descriptive references of mosquitoes are reproduced here as given by the authors, although in some instances they are probably not correct. For example, the recent publication of Cerqueira and Lane (62) on *capricornii* and that of Kumm, Osorno-Mesa, Boshell-Manrique (63) on Colombian species of *Haemagogus* indicate that the *Haemagogus* species designated by Bugher *et al* (31, 59) as *capricornii* is *H. spegazzinii* sub-species *jalcó*.

## DESCRIPTION OF COLLECTING STATIONS

Although some miscellaneous collecting was done in various localities in the *Study Area* and a good many animals, principally primates (marmosets), were purchased from local trappers, most of the collections were made at four stations. The selection of these stations was influenced by the human immunity surveys, the flora of the region and by transport facilities. It was desired to locate at least some of the stations where the virus had been present and where presumably there was a good chance of encountering it during the course of the study. In the beginning the only evidence in this regard was furnished by the diagnosed fatal infections and by human immunity surveys made prior to and during the incipency of the investigation. It was also desired to study and compare different ecological environments as reflected by the dominant character of the forest aggregates, and lastly it was essential to locate the stations where transportation of the collected material to the field laboratory at Pontal could be accomplished regularly and without great delay. Subsequently the location of one of the stations was dictated by the isolation of yellow fever virus from a marmoset captured by a local trapper. Immediately thereafter intensive collections were commenced in the locality and continued at intervals until the close of the study. The stations are designated: Fortuna, Pirataquicé, Urucutuca, and Almada, corresponding with the names of the fazendas, or nearest village.

Before proceeding to describe the collecting stations, it will be appropriate to indicate briefly the essential differences between old-or primary-type forest and young-type forest. The former possess a greater variety of species and fewer components of each species, while the latter or young-type forest is not so rich in variety of species but contains proportionally greater numbers of the species present. In addition the older forests have a higher and usually more homogenous and denser canopy of foliage which creates a more effective shade with the stunting or elimination of undergrowth. Another feature which characterizes and affects the environment in the older forests is the abundance of lianas and epiphytes in or immediately below the foliage canopy.

The *Fortuna station* located at the southern boundary of the *Study Area* was the first to be established. The location was chosen primarily because two fatal human infections were contracted in this vicinity in 1941 and also because it is on the northern edge of the massive climax forest that extends southward. The region is sparsely inhabited and is the nearest approach to a frontier zone. It is connected with Pontal by an unimproved dirt road which, however, can usually be negotiated by automobile. The small amount of cultivation, consisting of patches of mandioca and a few cacao plantations, and pasture land along the stream beds and narrow valleys, is of recent origin. Old or only slightly molested forests cover the high rolling hills. The dominant type of the forest aggregate at this station is primary in character. Both animals and mosquitoes were collected principally in the old-type forests, although some were collected along the periphery of a cacao plantation where there was an intermixture of second growth.



The station at *Pirataquicé*, located on a fazenda that has been exploited for many years, was the second to be established. It likewise was located in an extensive forested area due west of Ilhéus and south of the road leading to Itabuna. It is incorporated in the forested belt that runs along the eastern border of the *Study Area* and then widens to the south to include the Fortuna station. The forests at these two stations are similar in that they are predominantly of the old type, but the ones in *Pirataquicé* are megathermal-hygrophilous while at Fortuna they are mesothermal in character. The flatter terrain in this section permits more land to be under cultivation, mostly covered with older cacao plantations and some patches of mandioca and pasture fields. As at Fortuna, most of the captures were made in the older type of forest although some were made bordering on cacao groves where the trees are of younger age. Nonetheless, in analyzing the data it seemed justified to place the captures made at this, as well as the Fortuna station, in the category of old-type forests.

The station at *Urucutuca* was chosen because of an extensive swamp area that is under water during the more rainy months and has thus given rise to a special type of vegetation. In the flooded area the vegetation is low and aquatic in character but on more elevated patches of ground and about the edges other species are found. The patches of forest about the edge of the swamp are mostly second growth while those on the islands are representative of climax forest. It was principally on this higher land that the collections were made and, although the environment was no doubt affected to some degree by the proximity to the swamps, in certain group analyses the captures made here have been amalgamated with those made in second-growth or younger-type forests at the not far distant Almada station.

Collections at the *Almada station* were initiated following the isolation of virus from a marmoset and thereafter it was added to the regularly visited stations. Both *Urucutuca* and *Almada* are situated along the railroad line in the northeastern section of the *Study Area*. Nearly all of the arable land in this region is devoted to the cultivation of cacao and such forests as remain occur on hill tops, along stream beds, in swamps, or on permanently or periodically flooded land. These forests, with few exceptions, are of rather recent second growth because individual trees are cut as they reach a suitable size for fuel and for building purposes. While *Urucutuca* touches upon the narrowed belt of forest extending along the eastern border, *Almada* lies to the west although not far distant from this belt. As a result of the intensive cultivation of cacao this section is more densely populated than areas near Fortuna or *Pirataquicé*.

#### FIELD AND LABORATORY METHODS

The general plan was to collect at each station for a period of eight days then move on to the next station in sequence. As a day was required for assembling the traps, two days for rest of the field crew between each move, and a day in setting traps at the next location, a tour of all four stations was completed each 48 days. There were some exceptions to this rule, notably during the first stands at Fortuna and *Pirataquicé* which were somewhat longer, and at *Almada* following the isolation of virus. A part of the enlarged field crew was left at *Almada* and continuous captures of animals and mosquitoes were made in the vicinity for a period of four months.

In the beginning there was some experimenting done on the techniques of collecting but these were soon standardized, and in order to render the collections comparable the same kinds of traps, the same character of bait for trapping animals, and the same methods of capturing mosquitoes were employed at each station.

*Trapping of Mammals:* At each trapping station trails were cut through the forest and traps were set along either side of the trails at distances of 20 to 30 meters depending upon the availability of suitable sites. Most of them were set upon the ground but some were placed on logs, others on leaning tree trunks or in lianas at elevations of 1 to 2 meters. A few were located on tree platforms 10 or more meters above the ground.

Several sizes and types of traps were used, ranging from a tin-cap trap to a large collapsible wire trap. The one used most extensively was a rectangular wire mesh trap with a gravity-drop door and a false-floor trigger attachment. These traps have been described by Gilmore (72). Some Schuyler traps were employed, largely for exploratory trapping in relatively inaccessible places and for locating attractive sites and runways. The total number of traps in operation varied from 100 to 125.

Corn and bananas were most commonly used as bait. Papaya and other local fruits were used at times, and on occasion traps were baited with meat, usually bird or animal carcasses. The best results were obtained with well-ripened bananas.

The same trails were used during each successive visit to the station although the exact position of the traps varied. The traps were visited each morning, captured animals were removed and fresh bait was supplied. The animals were then sent to the field laboratory for measuring, weighing, species identification, withdrawal of blood and collection of ectoparasites.

As it was desired to obtain primates from different sections of the *Study Area*, the assistance of local trappers was solicited. Where active local agents could be found, the effort proved very successful and by far the larger number of marmosets was obtained by this means. Also a few primates were shot.

*Collection of Mosquitoes:* Mosquitoes were collected on the ground and at elevations in the trees varying from 10 to 20 meters depending upon the height and the character of the surrounding foliage. Platforms, reached by ladders, were constructed in some trees while others were scaled with climbing irons. Trees were selected which appeared to be most suitable for capturing *haemagogus* mosquitoes.<sup>7</sup> Captures were made in at least eight trees, approximately one hour being spent in each. The trees were situated in the vicinity of the trails where the animals were trapped, and the same ones were used during each tour of the station unless, due to poor captures, it was advisable to select a new tree.

Most of the captures were made free hand, the collector serving as bait. Care was taken to capture the mosquitoes immediately after they settled and before they had opportunity to draw blood. Any mosquitoes containing blood were discarded at the laboratory in order to avoid the possibility of the virus being neutralized, since all of the capturers were vaccinated against yellow fever. In the beginning Simplex suction tubes were employed, but this method was soon abandoned in favor of small flat-bottomed tubes, capped with snugly fitting inverted cups of Monel wire mesh. The bottom of each tube contained a layer of moistened blotting paper. The mosquitoes were captured individually in these tubes which were then placed in racks in a wooden box for transportation to the laboratory. This method avoided transfer of the mosquitoes and facilitated their classification in the laboratory. Also the mortality during transport was reduced to a minimum. The mosquitoes usually arrived at the laboratory within 24 hours after capture. Hourly records of the temperature and of the state of the weather were kept and the hour of capture was noted on the mosquito containers.

*Collection and Observation of Birds:* During the greater part of the study the information collected on birds was limited to observing the species present, their relative numbers,

<sup>7</sup> We are indebted to Dr. Jorge Boshell-Manrique, who visited the *Study Area*, for giving us some valuable advice on capturing *haemagogus* mosquitoes.

breeding habits, and evidence of migrations, etc. Only enough specimens were shot to verify their identity.

Later a technique was developed which proved satisfactory for obtaining specimens of blood and liver. The field equipment for this operation was carried in a knapsack and consisted of scissors, straight and artery forceps, shortened test tubes (16 X 35 mm.), rubber stoppers to fit, 2 and 5 cc. syringes with 20 or 22 gauge needles attached, all separately wrapped in paper and previously sterilized in the laboratory, cotton, a bottle of alcohol, and a wide-mouth thermos flask containing ice.

When a bird was shot it was immediately retrieved and was stunned by a blow on the head, if not already unconscious; the feathers over the chest were separated and the exposed skin was swabbed with alcohol. The chest wall was then opened with a pair of scissors exposing the heart, and while the assistant separated the chest wall with artery forceps, the needle with the syringe attached was plunged into the palpitating heart. The withdrawn blood was placed in one of the tubes. A section of the liver was then snipped off with another pair of sterile scissors and placed in a second tube. After being tightly stoppered the tubes were placed in the thermos and sent to the laboratory. Experience has shown that by using the proper bore gun and size of shot (No. 12 for small birds) an adequate quantity of sterile blood can be obtained from 80 per cent of the specimens.

#### LABORATORY PROCEDURES

It was a fixed rule that no virus should be retained at the Pontal laboratory. When the laboratory was established no yellow fever virus was taken there, and as soon as a virus was isolated and the preliminary neutralization test performed to determine whether or not it was that of yellow fever, it was immediately dispatched by air-express to Rio de Janeiro. Consequently, all of the neutralization tests on sera and further study on viruses isolated were made in the central laboratory at Rio de Janeiro. This procedure was followed, first and foremost, to avoid any possibility of an accidental contamination by a laboratory strain of virus being mistaken for the isolation of virus from captured arthropods and animals, and secondly, it was desired to perform no more work at the field laboratory than was essential.

*Mammals:* The animals captured at the different stations were brought to the laboratory for identification and to be tested for the presence of virus or antibodies in their sera. They were lightly anesthetized with ether, their ectoparasites were removed, and their weights and measurements recorded. The measurements taken included body length, tail length, length of foot and length of ear. These data were of aid in determining the species and the relative age of the animal. The sex was recorded and also the phase of the reproductive cycle if the animal was a female. The skin and skulls of all animals about which there existed any question of identity were preserved for future reference. In addition skin and skull specimens of approximately 60 per cent of all animals captured were prepared and subsequently presented to the National Museum of Natural History at Rio de Janeiro.

A blood specimen was obtained from each animal and the separated serum placed in ampules was sent to the Rio de Janeiro laboratory for a neutralization test. In addition the blood from animals other than marmosets was tested for the presence of yellow fever virus by subinoculating the serum into non-immune marmosets. As the supply of marmosets did not permit subinoculating the serum from individual animals, the sera from animals of the same species were pooled. The marmosets inoculated were tested at intervals for circulating virus and the development of immunity as later described.

The blood serum from marmosets was not routinely subinoculated, as it had been established that marmosets either die of the infection or develop neutralizing antibodies. The marmosets were bled on receipt at the laboratory in order to secure serum for immunity tests, and were kept under observation until the result of this test became known, usually for a minimum period of two weeks. A majority of the non-immune animals were used

eventually for experimental injections. Before use they were rebled and the second serum specimen tested. This procedure was employed to detect possible infection prior to capture, at a time so recent that antibodies had not developed when the marmoset was first bled. If a marmoset died during this observation period, it was autopsied and if the findings suggested yellow fever, passages to mice were made of its serum and liver tissue. A portion of the liver and also a sample of the serum were preserved by desiccation from the frozen state. Portions of the more important organs of all marmosets dying while under observation were prepared for histopathological examination.

**Mosquitoes:** When field collections of mosquitoes were received at the laboratory, the insects were identified individually and recorded by genera or species and place of capture. The insects collected on each day were then pooled, by genus or by species, into four groups. Group A was composed of all of the mosquitoes included in the genera *Trichoprosopon*, *Wyeomyia*, *Phoniomyia*, *Limatus* and *Sabethes*. Group B included all of the mosquitoes in the two genera *Aedes* and *Psorophora*. Group C comprised all of the *Chagasia*, *Anopheles*, *Tacniorhynchus* and *Culex*. Group D included the genus *Haemagogus* only.

After being combined into these groups the mosquitoes were killed by exposure to cyanide fumes. The insects of each group were then ground in a mortar without abrasive and suspended in a serum-saline solution in proportion of 0.05 ml., for each mosquito. The serum-saline solution used here and referred to elsewhere consisted of one part of normal rhesus monkey serum in nine parts of 0.9 per cent NaCl solution. If there were less than 20 insects in any group, a minimal quantity of 1.0 ml., of diluent was used. The mosquito emulsion was centrifuged for 20 minutes at a speed of 2000 r.p.m. The resulting supernatant was injected either into a rhesus monkey or into a marmoset. The total volume of the supernatant was inoculated provided it did not exceed 3.0 ml., which for reasons of toxicity was the maximum given. The inoculated animals were bled every three days and their sera were injected into mice to test for the presence of virus. Separate animals were used for each collecting station and for each mosquito group. The animal received successive daily inoculations during the collecting period at a given station. Ten to 14 days after the termination of the series of injections the animal was bled again and the serum sent to Rio de Janeiro to be tested for the presence of neutralizing antibodies.

**Ectoparasites:** Ectoparasites were collected by wrapping the animal in a white cloth, placing it in a jar containing ether and when the animal was under light anesthesia removing it and combing its fur while holding it over the cloth. Any parasites present about the ears were removed with forceps. All parasites collected during the first two months of the study were preserved for identification; those collected in the later months were tested for the presence of virus. All parasites obtained from rodents on any one day were combined into one group, those collected from marsupials formed a second group. The parasites were triturated and a 10 per cent suspension was made in serum-saline solution. Following centrifugation of the suspension at 2000 r.p.m. for 10 minutes, two marmosets were injected with the supernatant, a volume of 1.0 ml., being used for each inoculum. The same two marmosets were used for repeated injections of parasites derived from the same order of animals during a collecting period. The marmosets were tested for circulating virus and for immunity according to the methods described for monkeys injected with mosquito emulsions.

**Birds:** The liver specimens taken in the field were triturated and a 10 per cent suspension was made in serum-saline solution. Following light centrifugation the supernatant was inoculated into a group of six white mice. Each mouse received 0.03 ml., intracerebrally. The blood serum was subjected to the neutralization test.

**Identification of Virus:** If any mouse became ill, following the injection of material being tested for the presence of virus, it was sacrificed and the brain was removed under aseptic precautions. The brain was triturated in sufficient saline to make a 10 per cent concentration by weight and the suspension centrifuged at 2000 r.p.m. for 20 minutes. The supernatant was used in a specificity test designed to determine whether or not the in-

fectious agent was yellow fever virus. A portion of the supernatant was also preserved by desiccation from the frozen state.

As previously stated, all viruses isolated were sent to the Central Laboratory at Rio de Janeiro for confirmatory identification and further study. All of the infectious material at the field laboratory was then destroyed.

*Neutralization Tests:* Blood samples for the neutralization test were collected as above described. The sera were separated, placed in ampules, sealed and sent by air-express to the laboratory at Rio de Janeiro where they were tested according to the technique described in Part I.

## RESULTS

### *Ecology*

This does not presume to be an ecological study in the general application of the term. Only such information was collected as would seem to have direct bearing upon the epidemiology of jungle yellow fever. The following data which have been placed under the caption of ecology are confined to the relative prevalence of animals and mosquitoes in the different types and levels of sylvan vegetation, and to such information as was obtained on the breeding season of animals and the quantitative seasonal fluctuations in certain species of mosquitoes.

Any method of collecting animals is necessarily selective and usually does not reflect the actual prevalence of the different species inhabiting the forests. However, it is usually possible to obtain a fairly accurate comparison of the animals in different types of forests by using the same traps and the same kind of bait at all collecting stations. Marmosets and perhaps other arboreal animals may constitute an exception to this rule as it is much more difficult to trap marmosets in old extensive forests than in cacao groves and small patches of second-growth forests. This is due to their feeding habits and obstacles encountered in placing traps at attractive sites in the old high forests. Also, marmosets can be observed with greater facility in small patches of forests and their excursions into cacao groves noted, thus making it possible to set the traps in places they tend to frequent.

A total of 5,322 mammals was collected, consisting of the following orders: Rodentia 2,300 (43.2 per cent); Primata 1,851 (34.8 per cent); Marsupialia 1,040 (19.5 per cent); Chiroptera 81 (1.5 per cent); Carnivora 28 (0.5 per cent); Edentata 21 (0.4 per cent) and one specimen of Artiodactyla. As stated above most of the collections, with the exception of primates, were made at the four stations. The total number of "trap-nights" of all types of traps at each of these four stations was: Pirataquicé 11,455, Fortuna 7,644, Almada 7,969, Urucutuca 5,840, making a grand total of 32,908. The trap-nights were calculated by multiplying the number of traps by the number of nights the traps were set.

A list of the animals classified by genera or species and by the type of vegetation at the site of capture are presented in Tables 5 and 7. Consideration of the breeding seasons and relative quantity of mammals in different environments

will be limited to rodents, marsupials and primates, since these are the only orders about which adequate data were obtained. Collections made at the Pirataquicé and Fortuna stations are grouped (Table 5) under "Old-type forest" and those made at Almada and Urucutuca are grouped under "Young-type forest" or "Swamp forest." Significant differences between the captures at each of the four stations will be noted in the text.

*Rodents:* The genus *Oryzomys* accounted for 57.7 per cent of all rodents captured. The six most common species were *Oryzomys oniscus* (38.2 per cent), *Nectomys squamipes* (13.8 per cent), *Oryzomys* sp. (11.6 per cent), *Akodon nigrita* (11.1 per cent), *Akodon arviculoides* (9.0 per cent), and *Oryzomys eliurus* (7.9 per cent). The remaining 14 identified species were found in relatively small numbers (Table 5).

Notable deviations among the species captured in different types of forests are: *N. squamipes*, though found at all stations near streamlets, was most frequent in swamp forests (Urucutuca); *O. oniscus* was found in greater numbers in old-type forests, whereas *O. eliurus* was found largely in young-type forests; *A. nigrita* is a common inhabitant of old-type forests and rare in swamp forests, but the other species of this genus, *A. arviculoides*, is common in both young-type and swamp forests and rare in old-type forests. *Proechimys setosus* was found almost exclusively (33 of 34 specimens) in the old-type forest at Fortuna.

Judged by the capture of pregnant females and young specimens, there are two principal breeding seasons, one from January to May and the other from August to November. No pregnant females and few young were taken during the months of June, July and December. The highest percentage of young were captured in April and May.

*Marsupials:* The five more common species, constituting 94.3 per cent of 1,040 marsupials captured are listed below. (See also Table 5.)

	Per cent
<i>Metachirus nudicaudatus</i> .....	27.1
<i>Didelphis marsupialis</i> .....	23.8
<i>Marmosa cinerea</i> .....	17.1
<i>Marmosa murina</i> .....	13.7
<i>Marmosa incana</i> .....	12.6

A few *Marmosa agilis* (3.9 per cent) and *Monodelphis americana* (1.6 per cent) and one each of *Chironectes minimus* and *Metachirops opossum* were also captured.

*Metachirus nudicaudatus* and *Didelphis marsupialis* comprised most of the animals captured in old-type forests. With the exception of *M. cinerea*, few other species of *Marmosa* were obtained at these stations. On the other hand, *Marmosa* were quite frequent in young-type and swamp forests. No specimens of *Metachirus* were trapped in swamp forest.

Females with pouch-young were captured during each month of the year, but the principal breeding season begins in September and extends through March. During the remaining months of the year, the breeding is much reduced.

The number of rodents and marsupials captured per 100 trap-nights and with

TABLE 5

List of mammals collected (excluding primates), classified according to order, genus or species, and type of vegetation where captured

ORDER, GENUS OR SPECIES	TYPE OF VEGETATION									
	Old-type Forest		Young-type Forest		Swamp Forest		Miscellaneous		Grand Total	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
<b>Marsupialia</b>										
<i>Didelphis marsupialis</i> L.	156	32.4	57	14.0	19	31.1	15	16.5	247	23.8
<i>Metachirops opossum</i> (L.)	1	0.2							1	0.1
<i>Metachirus nudicaudatus</i> (E. Geoffroy)	206	42.7	62	15.3			14	15.4	282	27.1
<i>Chironectes minimus</i> Zimmermann							1	1.1	1	0.1
<i>Marmosa cinerea</i> (Temminck)	61	12.7	85	20.9	10	16.4	22	24.2	178	17.1
<i>Marmosa murina</i> (L.)	26	5.4	88	21.7	10	16.4	18	19.8	142	13.7
<i>Marmosa incana</i> (Lund)	6	1.2	102	25.1	19	31.1	4	4.4	131	12.6
<i>Marmosa agilis</i> Burmeister	16	3.3	6	1.5	3	4.9	16	17.6	41	3.9
<i>Monodelphis americana</i> (Müller)	10	2.1	6	1.5			1	1.1	17	1.6
<b>Total Marsupialia</b>	<b>482</b>	<b>100.0</b>	<b>406</b>	<b>100.0</b>	<b>61</b>	<b>99.9</b>	<b>91</b>	<b>100.1</b>	<b>1040</b>	<b>100.0</b>
<b>Rodentia</b>										
<i>Rattus rattus rattus</i> (L.)	4	0.3	1	0.1					5	0.2
<i>Rattus rattus frugivorus</i> Raf.	3	0.2	1	0.1			2	2.1	6	0.3
<i>Oecomys cinnamomeus</i> (J. Pict. & C. Pict.)	3	0.2	12	1.6	1	0.7	13	13.7	29	1.3
<i>Oryzomys elurus</i> (Wagner)	10	0.8	163	21.4	6	4.1	3	3.2	182	7.9
<i>Oryzomys oniscus</i> (Thomas)	700	54.1	147	19.3	23	15.5	8	8.4	878	38.2
<i>Oryzomys sp.</i>	146	11.3	95	12.5	11	7.4	14	14.7	266	11.6
<i>Nectomys squamipes</i> Brants	146	11.3	84	11.0	77	52.0	11	11.6	318	13.8
<i>Rhipidomys maculipes</i> (J. Pict. & C. Pict.)	11	0.9	31	4.1	5	3.4	6	6.3	53	2.3
<i>Akodon arviculoides</i> (Wagner)	11	0.9	165	21.6	21	14.2	10	10.5	207	9.0
<i>Akodon nigrita</i> (Licht.)	192	14.8	58	7.6	2	1.4	3	3.2	255	11.1
<i>Blarinomys breviceps</i> (Winge)	5	0.4					2	2.1	7	0.3
<i>Phyllomys blainvillei</i> (Jourdan)	3	0.2	1	0.1	1	0.7			5	0.2
<i>Phyllomys brasiliensis</i> Waterhouse	5	0.4							5	0.2
<i>Isothrux picta</i> (Pictet)			1	0.1			2	2.1	3	0.1
<i>Proechimys setosus</i> (Desm.)	33	2.6	1	0.1					34	1.5
<i>Coendou melanurus</i> (Wagner)	2	0.2					4	4.2	6	0.3
<i>Chactomys subspinosus</i> (Kuhl)	3	0.2					11	11.6	14	0.6

TABLE 5 (Concluded)

ORDER, GENUS OR SPECIES	TYPE OF VEGETATION									
	Old-type Forest		Young-type Forest		Swamp Forest		Miscellaneous		Grand Total	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
<b>Rodentia—Continued</b>										
<i>Dasyprocta aguti</i> (L.)....	5	0.4					2	2.1	7	0.3
<i>Galca wellsi</i> (Osgood)....			1	0.1			3	3.2	4	0.2
<i>Guerlinguetus ingrami</i> Thomas.....	12	0.9	2	0.3	1	0.7	1	1.1	16	0.7
<b>Total Rodentia.....</b>	<b>1,294</b>	<b>100.1</b>	<b>763</b>	<b>100.0</b>	<b>148</b>	<b>100.1</b>	<b>95</b>	<b>100.1</b>	<b>2,300</b>	<b>100.1</b>
<b>Carnivora</b>										
<i>Procyon cancrivorus</i> v. Ihering.....	1	5.0							1	3.6
<i>Potos flavus</i> (Schreber)...	17	85.0							17	60.7
<i>Tayra barbara</i> (L.).....	1	5.0							1	3.6
<i>Grisson vittatus</i> (Schreber)							3	50.0	3	10.7
<i>Dusicyon</i> (C.) <i>thous</i> (Wied).....	1	5.0	2	100.0			3	50.0	6	21.4
<b>Total Carnivora.....</b>	<b>20</b>	<b>100.0</b>	<b>2</b>	<b>100.0</b>			<b>6</b>	<b>100.0</b>	<b>28</b>	<b>100.0</b>
<b>Edentata</b>										
<i>Scacopus torquatus</i> (Ill.)...	5	55.6			1	100.0	2	20.0	8	38.1
<i>Tamandua tetradactyla</i> (L.).....							7	70.0	7	33.3
<i>Dasipus novemcinctus</i> L.	4	44.4	1	100.0			1	10.0	6	28.6
<b>Total Edentata.....</b>	<b>9</b>	<b>100.0</b>	<b>1</b>	<b>100.0</b>	<b>1</b>	<b>100.0</b>	<b>10</b>	<b>100.0</b>	<b>21</b>	<b>100.0</b>
<b>Chiroptera</b>										
<i>Glossophaga soricina</i> (Pallas).....	7	38.9	11	30.6			1	4.5	19	23.5
<i>Lonchophylla mordax</i> Thomas.....	3	16.7	4	11.1	1	20.0	6	27.3	14	17.3
<i>Lonchoglossa ecaudata</i> (Spix).....			2	5.6			3	13.6	5	6.2
<i>Carollia perspicillata</i> (L.)			7	19.4	1	20.0	2	9.1	10	12.3
<i>Artibeus p. planirostris</i> (Spix).....							2	9.1	2	2.5
<i>Molossus rufus</i> (Geoffroy)			7	19.4			5	22.7	12	14.8
<i>Molossus obscurus</i> (Geoffroy).....			1	2.8					1	1.2
Not classified.....	8	44.4	4	11.1	3	60.0	3	13.6	18	22.2
<b>Total Chiroptera.....</b>	<b>18</b>	<b>100.0</b>	<b>36</b>	<b>100.0</b>	<b>5</b>	<b>100.0</b>	<b>22</b>	<b>99.9</b>	<b>81</b>	<b>100.0</b>
<b>Artiodactyla</b>										
<i>Tagassu tajacu</i> L.....	1								1	
<b>Grand total.....</b>	<b>1,824</b>		<b>1,208</b>		<b>215</b>		<b>224</b>		<b>3,471</b>	



different types of traps at each of the four regular collecting stations is shown in table 6.

As the ratio of trap-nights of the different types of traps employed was not exactly the same for each station and as there was a marked variation in the number of rodents and marsupials captured in the different types of traps, the

TABLE 6

*Captures of rodents and marsupials per 100 trap-nights by station and type of trap*

STATION	FORTUNA		PIRATAQUICÉ		ALMADA		URUCUTUCA		TOTAL	
Trap-nights by type of trap										
Collapsible wire...	1647		2736		1503		1056		6942	
Rectangular wire..	2920		3680		3026		2136		11762	
Tin can.....	1439		2377		1415		1152		6383	
Ratoeira and Schuyler.....	1638		2662		2025		1496		7821	
Total.....	7644		11455		7969		5840		32908	
CAPTURES BY TYPE OF TRAP	RO-DENTS	MAR-SUPIALS	RO-DENTS	MAR-SUPIALS	RO-DENTS	MAR-SUPIALS	RO-DENTS	MAR-SUPIALS	RO-DENTS	MAR-SUPIALS
Collapsible wire....	5	14	28	124	1	27	0	15	34	180
Captures per 100 trap-nights.....	.30	.85	1.02	4.53	.07	1.80	0	1.42	.49	2.59
Rectangular wire....	280	85	638	144	250	141	356	92	1524	462
Captures per 100 trap-nights.....	9.59	2.91	17.34	3.91	8.26	4.66	16.67	4.31	12.96	3.93
Tin can.....	125	5	148	11	34	15	144	14	451	45
Captures per 100 trap-nights.....	8.69	.35	6.23	.46	2.40	1.06	12.50	1.22	7.08	.70
Schuyler.....	5	9	8	25	15	31	4	16	32	81
Captures per 100 trap-nights.....	.31	.55	.30	.94	.74	1.53	.27	1.07	.41	1.04
Total.....	415	113	822	304	300	214	504	137	2041	768
Captures per 100 trap-nights.....	5.43	1.48	7.18	2.65	3.76	2.69	8.63	2.35	6.20	2.33
Ratio rodents/marsupials.....	3.67		2.70		1.40		3.68		2.66	

ratios of total captures per 100 trap-nights for all traps as shown in the bottom row of the table are not strictly comparable. It would therefore be more accurate to compare the captures according to types of trap in which these animals were commonly captured. The rodents were captured principally in the rectangular wire and tin can traps, while the marsupials were taken largely in the rectangular and collapsible wire traps. The fluctuations in the captures at different stations relative to the type of trap is in part conditioned by the size.

of the species most common in the area. For example, where the dominant species of rodents were small in size more captures of this order were made in the tin can traps and when the larger species of marsupials prevailed proportionally more of this order were collected in the large collapsible wire traps. If the frequency of capture is accepted as a measure of prevalence, it may be inferred that the rodents were most numerous at Urucutuca and Pirataquicé and least at the Almada station, and that the marsupials were present in order of abundance at Almada, Pirataquicé, Urucutuca, and Fortuna. The relative predominance of rodents over marsupials was greatest at Urucutuca and Fortuna and least at Almada.

*Primates:* The number of primates collected during the course of study amounted to 1,851, the great majority of which (1,829) were marmosets of the

TABLE 7

*List of primates collected, classified according to species and type of vegetation where captured*

TYPE OF VEGETATION	<i>Leontocbus chrysomelas</i> (Kuhl)	<i>Callithrix penicillata</i> (E. Geoffroy)	<i>Callicebus gigot</i> (Spix)	<i>Alouatta guariba</i> (Humb.)	<i>Cebus xan- thoesternus</i> Wied	TOTAL
Old-type forest.....	7	155		3	2	167
Young-type forest.....	1	590				591
Swamp forest.....	1	15	1			17
Cacao plantation.....		936				936
Banana patch.....		26				26
Coconut grove.....		62				62
Not specified.....	5	45	1		1	52
Total.....	14	1829	2	3	3	1851

species *Callithrix penicillata*. Of the remaining species, there were 14 *Leontocbus chrysomelas*, three *Alouatta guariba*, three *Cebus xanthoesternus*, and two *Callicebus gigot*. (Table 7.)

The procedure employed in procuring primates, particularly marmosets (*C. penicillata*), does not permit an accurate determination of their relative abundance in different localities. The great majority (83 per cent) was purchased through local agents, and the number captured in any given district depended to a large degree upon the energy and ability of the agent in stimulating the local inhabitants to trap them. In some of the more inaccessible parts it was not feasible to secure satisfactory agents or to arrange for the delivery of the captured animals to the laboratory. Consequently, the sampling was spotty and irregular. There is little doubt, however, that this species is widely distributed throughout the region and the number captured in localities where trapping was thorough indicates that they exist in great plenty.

The largest numbers were trapped in cacao plantations and in the forest of limited extent. This may be partially accounted for by the fact that trapping in districts devoted to cacao production, and does not necessarily imply that cacao groves constitute a permanent habitat. It is a common observation

that marmosets dwelling in a bordering forest enter cacao groves to feed in the early morning hours, and they can be trapped here with greater facility than in the forest. Some may live permanently in the heavily shaded cacao groves where foliage of large trees of other varieties form a more or less contiguous upper canopy.

The other species of primates are much less numerous and were found principally in old extensive forests. Their distribution is therefore limited to areas where this type of vegetation exists.

*C. penicillata* breed to some extent throughout the year but the greatest number of pregnant females was encountered during the months of September, October and November.

*Birds:* During the course of observation on birds 45 families and 196 species were recognized. Members of the following five families were most frequently observed: *Thraupidae*, *Tyrannidae*, *Icteridae*, *Fringillidae*, *Hirundinidae*.

The five most common species were: *Ramphocelus bresilius*, *Cacicus cela*, *Jacana spinosa*, *Turdus rufiventris*, *Stelgidopteryx ruficollis*.

In general birds were more abundant at Pirataquicé, followed by Almada, Fortuna and Urucutuca in the order named.

The principal breeding season extended from August to February.

Very little is known concerning the migration of forest birds in tropical Brazil nor did these observations add any significant information to this question. All that can be said is that few, if any, birds known to possess migratory habits, at least in this region, were recognized.<sup>8</sup>

*Arthropods:* The collection of arthropods was confined almost exclusively to ectoparasites and mosquitoes, as they were considered to be the ones most likely involved in the transmission of the virus. Moreover, other haemaphagous arthropods were observed in very limited numbers, consisting principally of a few *Tabanida* and *Simulidae*.

1. *Ectoparasites:* Ectoparasites comprising the families *Pulicidae*, *Ixodidae* and *Laelaptidae* were found in greatest abundance on rodents. *Laelaptidae* were consistently present and in numbers greater than the other two families combined. The average number of ectoparasites found on 300 rodents of the genus *Oryzomys* was 27, and on 138 *Nectomys* 15. In each instance the *Laelaptidae* constituted over 95 per cent of the parasites. Parasites were present, but less numerous, on other genera of rodents.

Marsupials were also hosts of ectoparasites. The *Pulicidae* and the *Ixodidae* were found more frequently than *Laelaptidae* although when the latter family occurred there was usually a considerable number of them. The average number of ectoparasites on marsupials varied according to species from two to eight.

It is significant that ectoparasites were found only rarely upon primates.

2. *Mosquitoes:* As certain *Culicidae* had been shown to be vectors of the virus, this family of insects received special attention. While the main effort

<sup>8</sup> More complete data on birds in this region may be published by Pedro Britto who was responsible for making the field observations.

was directed to the isolation of the virus from mosquitoes, some ecological data, related to habitat of the different genera and species, were obtained.

The relative prevalence of genera or species in different types of vegetation and at ground and upper vegetational levels is shown in table 8. These data are based upon the capture of 87,583 mosquitoes during 4,717 man-hours. As previously described, the captures were made free hand, the collector serving as bait. The total number of mosquitoes captured per 100 man-hours was highest in young-type forests and lowest in swamp forests. This is somewhat surprising as swamps are commonly believed to harbor a high density of mosquitoes. It should be remembered, however, that the captures were made in forest growth and that the rainfall in this region is heavy and fairly evenly distributed throughout the year. Even in the upland forests the humidity is relatively high and there is usually enough collection of water on the ground (streams) and in the trees (holes, etc.) to serve for breeding. Only three species, captured in significant numbers, *Aedes scapularis*, *Taeniorhynchus fasciolata* and *T. chrysonotum*, were more numerous in swamp than in other types of forests. *Trichoprosopon*, *Psorophora albipes* and *Aedes fulvus* were notably more frequent in young-type forests than in old-type forests, the latter two occurring in very small numbers at the Fortuna station. On the other hand *Phoniomyia*, *Sabethes*, *Limatus* and *Haemagogus* are definitely more prevalent in the old-type forests than in either young-type or swamp forests. Of these all but *Limatus* were captured in larger numbers in the trees than upon the ground, irrespective of the nature of the forest. Other mosquitoes taken in significant numbers were consistently more abundant at the ground level than in the trees. *Psorophora albipes* is a partial exception to this rule in that in young-type and swamp forests the captures in the trees were slightly in excess of the ground captures but in the older forests more were found at the ground level.

A few crepuscule and night captures were made in order to ascertain if any species were being missed by making captures only during the brighter hours of the day. While a few mosquitoes, notably *Trichoprosopon*, *Psorophora cingulata*, *Aedes fulvus*, *Anopheles mediopunctatus*, *Taeniorhynchus chrysonotum* and *T. fasciolata* were collected somewhat more frequently at night than other species, no species was taken at night that was not also captured during the day. Conversely, there were several genera and species that were captured only during the day, for example *Sabethes*, *Haemagogus* and some of the rarer *Aedes*.

In that the genus *Haemagogus* has been most frequently identified as a vector of the virus in nature, data collected on the environment and feeding habits of this mosquito were further analyzed. Apparently the *Haemagogus* mosquitoes in this area are predominantly, if not entirely, *H. spegazzinii* as all of the males reared from captured females were identified as this species. It has been noted that this mosquito is captured more frequently in old-type forests and at upper vegetational levels. Some exploratory captures were also made in cacao groves and *Haemagogus* was found to be present in about the same numbers as in neighboring young-type forests. It was somewhat more abundant in groves

TABLE 8

Classification of mosquitoes, showing number of specimens captured per 100 man-hours, in relation to type of vegetation and capture level

GROUPS BY GENUS OR SPECIES	OLD-TYPE FOREST						YOUNG-TYPE FOREST		SWAMP FOREST		TOTAL	
	Pirataquicé		Fortuna		Total							
	Ground	Tree	Ground	Tree	Ground	Tree	Ground	Tree	Ground	Tree	Ground	Tree
<i>Trichoprosopon</i> .....	423	16	402	47	413	30	824	19	70	6	483	22
<i>Wyeomyia</i> .....	1145	34	1201	34	1173	34	888	27	364	70	893	36
<i>Phoniomyia</i> .....	107	230	252	768	180	476	14	101	73	185	97	269
<i>Limatus</i> .....	568	2	592	2	580	2	317	7	30	1	365	4
<i>Sabethes</i> .....	18	251	29	204	24	229	23	40	8	16	20	116
<i>Psorophora albipes</i> .....	218	68	8	1	113	37	854	994	413	538	442	531
<i>Psorophora ferox</i> .....	92	9	120	7	106	8	49	5	12	1	65	6
<i>Psorophora lutzii</i> .....	8		4		6		6	*	35	6	13	1
<i>Psorophora cingulata</i> ....	4	*	2		3	*	15	1	5	*	8	*
<i>Aedes fulvus</i> .....	81	3	6		43	1	116	4	27	3	66	3
<i>Aedes nubilus</i> .....	19	*	3		11	*	9	*	*		8	*
<i>Aedes scapularis</i> .....	26	1	4	*	15	1	28	2	185	7	57	2
<i>Aedes serratus</i> .....	475	18	152	5	313	12	382	20	110	10	292	15
<i>Aedes fluviatilis</i> .....	*				*						*	
<i>Aedes terrens</i> .....	1		2		1						1	
<i>Aedes fulvithorax</i> .....	*		2		1		*		*		1	
<i>Aedes (Howardina) sp.</i> ....	1	*	4	*	3	*	1	*			1	*
<i>Chagasia fajardoi</i> .....	*	4	*	5	*	4					*	2
<i>Anopheles nimbus</i> .....	1				*		3	*	7	*	3	*
<i>Anopheles fluminensis</i> ...	1				*		*				*	
<i>Anopheles intermedius</i> ...							2	*	3	*	1	*
<i>Anopheles mediopunctatus</i>	2				1		2	*	4	1	2	*
<i>Anopheles tarsimaculatus</i>												
group.....	*				*		*		6	1	1	*
<i>Anopheles triannulatus</i> ...								*	1		*	*
<i>Anopheles bellator</i> .....	1	3	4	27	2	14	*	2	*	*	1	7
<i>Anopheles cruzi</i> .....		*	*	1	*	1		*			*	*
<i>Taeniorhynchus</i> .....	2	*			1	*					*	*
<i>Taeniorhynchus</i>												
<i>indubitans</i> .....		*	3	*	1	*	1	1	1	4	1	1
<i>Taeniorhynchus albicosta</i> .									2	*	1	*
<i>Taeniorhynchus</i>												
<i>chrysonotum</i> .....	4	*			2	*	20	4	65	13	22	4
<i>Taeniorhynchus fasciolata</i>	18	*	1		10	*	42	2	238	21	72	4
<i>Culex</i> .....	1	*			*	*	9	2	15	9	7	2
<i>Aedes leucocelaenus</i> .....	*	4	3	4	1	4		1			1	2
<i>Haemagogus</i> .....	34	380	37	248	36	320	18	67	2	4	22	164
Total captured.....	12253	6816	10765	7558	23018	14374	23019	16855	6619	3698	52656	34927
Total man hours.....	377	665	380	558	757	1223	635	1296	395	411	1787	2930
Total per 100 man-hours.....	3250	1025	2833	1355	3041	1175	3625	1301	1676	900	2947	1192

\* Less than one.

well shaded with other species of trees. Here, as elsewhere, more of the mosquitoes were captured in the trees than at ground level. They were rarely found in swamp forest.

*Haemagogus* is strictly a day feeder and the heaviest captures were made between the hours of 9 a.m., and 2 p.m., irrespective of the type of vegetation

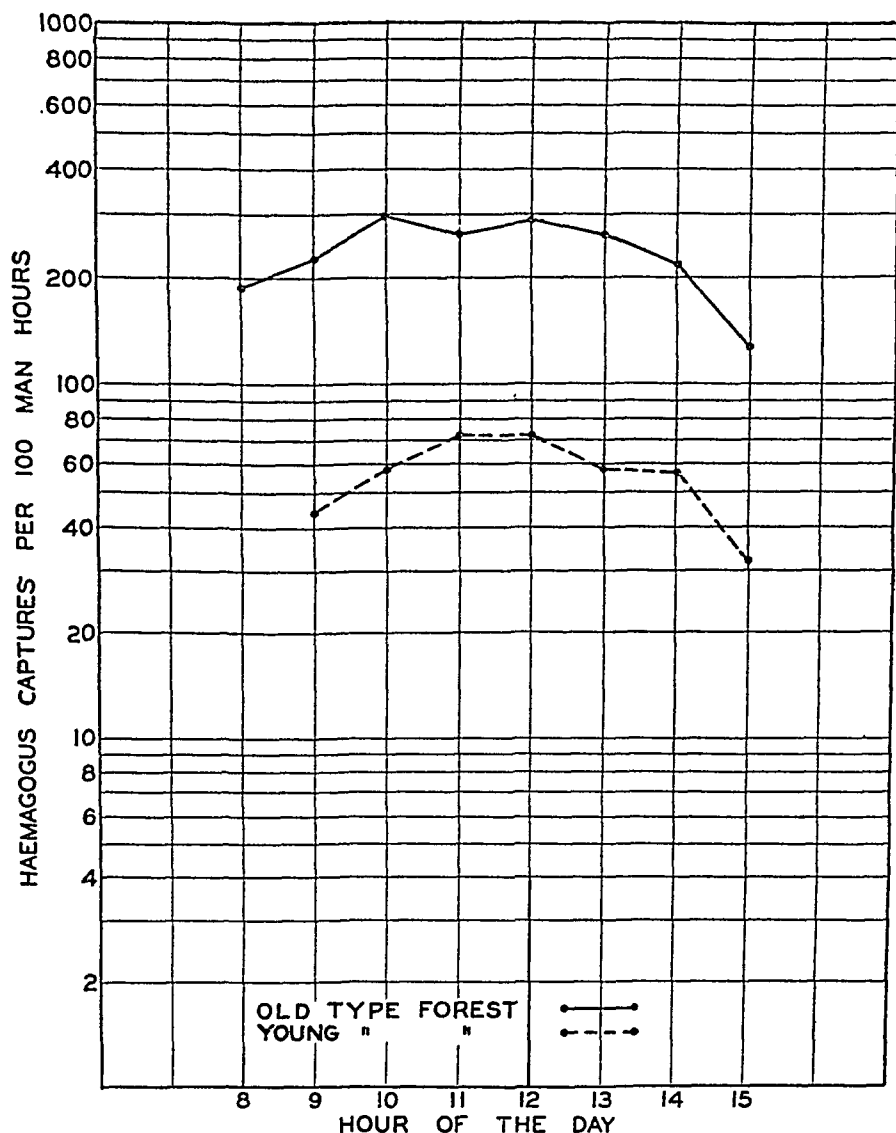


FIG. 2. Capture of haemagogus mosquitoes per 100 man-hours in old-type and in young-type forests at different hours of the day.

(fig. 2). In fig. 3 these data have been further broken down into ground and tree captures. It is evident that the prevalence of *Haemagogus* as determined by the capture of females in search of a blood meal is influenced by the type of forest, the vegetational level and the hour of the day the captures are made. These characteristics have been observed by Bugher *et al.* (31), Boshell-Manrique and Osorno-Mesa (59), and by Bates (73) who has made a careful study

of the microclimate favored by *Haemagogus*. Although it is definitely photophilous and is found in largest numbers in trees over which the foliage canopy is not so dense as to obscure the sky entirely, in our experience it was captured in greater numbers during partially cloudy hours than during bright sunshine. This difference was especially manifest in tree captures and during the forenoon.

*Haemagogus* was present during all seasons of the year. The following tabulation represents the captures made at the Pirataquicé station:

	NUMBER CAPTURED	NUMBER MAN-HOURS	NUMBER PER 100 MAN-HOURS
1944			
May.....	653	82	796
July.....	252	92	274
September.....	443	112	396
November.....	242	112	216
December.....	417	81	515
1945			
February.....	365	83	440
March.....	158	103	153
Total.....	2530	665	380

The variations in the number captured at different periods may have been influenced to some degree by the weather conditions at the time the captures were made. However, the collections do reflect the relative number of females in search of a blood meal, which as far as virus transmission is concerned is perhaps more important than their actual prevalence at any given time.

#### *Neutralization Tests on Captured Mammals*

*Non-specific Reactions:* In evaluating the significance of the neutralization test, consideration should be given to apparently non-specific reactions which are not infrequently encountered among certain species of animals.

To ascertain if this applied to rodents and marsupials in the Ilhéus region, 211 rodent and 19 marsupial sera which gave a positive neutralization test against yellow fever virus, were tested against Japanese B virus. The test was performed in a manner similar to that described for yellow fever virus, with the exception that a greater amount of virus, approximately 1,000 MLD, was employed. It was found that 188 of 211 (89 per cent) of the rodent and 10 of 19 marsupial sera, partially or completely inactivated the Japanese B virus. This non-specific behavior is further attested by the neutralization of yellow fever virus by sera of rodents and marsupials captured in regions in which there is every reason to believe that yellow fever does not exist. Aging of the serum may also be a factor in these non-specific reactions in rodents and marsupials as it was found that some sera, though negative when originally tested, gave a positive reaction when tested some months later.

Information on non-specific reactions in birds is lacking. The few bird sera collected during the study were insufficient in quantity to permit testing against

other viruses. It is suspected, however, that non-specific reactions may occur among avian species since positive neutralization tests have been observed in sera from birds that probably had had no contact with the virus.

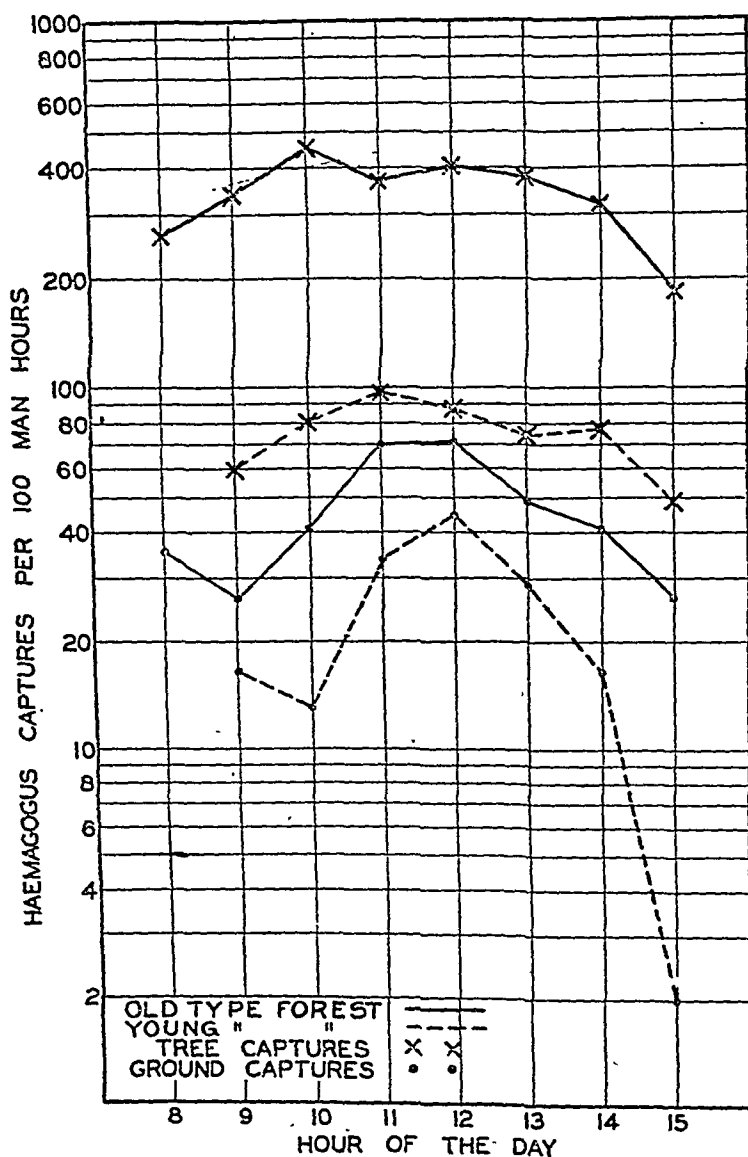


FIG. 3. Capture of haemagogus mosquitoes per 100 man-hours in old-type and in young-type forests, on ground and in trees at different hours of the day.

On the other hand, false positives are very rarely encountered in marmosets and other primates, nor does aging of the serum seem to exert any influence. Of the 99 marmoset (*Callithrix penicillata*) sera that neutralized yellow fever virus, 87 were tested against Japanese B virus, and only two manifested virucidal action, in one of which the inactivation was inconclusive. Furthermore, none of the several hundred sera of a closely related species (*Callithrix aurita*) captured in the vicinity of Rio de Janeiro neutralized yellow fever virus.



Another factor which must be considered is the duration of the immunity. Observations made in this laboratory imply that in some of the marsupials neutralizing antibodies to yellow fever virus may be lost after a period of several months. It was found that seven of 22 *Metachirus nudicaudatus* which had been artificially infected with yellow fever virus and had given a positive neutralization test three weeks following infection failed to neutralize the virus

TABLE 9

## RODENTS

Results of neutralization tests with yellow fever virus, with species listed according to type of vegetation in which they were captured

SPECIES	TYPE OF VEGETATION											
	Old-type forest			Young-type forest			Swamp forest			Grand Total		
	Pos.	Total	Per cent Pos.	Pos.	Total	Per cent Pos.	Pos.	Total	Per cent Pos.	Pos.	Total	Per cent Pos.
<i>Guerlinguetus ingrami</i> .....		2						1			3	
<i>Coendou melanurus</i> .....		1									1	
<i>Chaetomys subspinosus</i> .....		1									1	
<i>Galea welsi</i> .....		3									3	
<i>Proechimys setosus</i> .....		31			1						32	
<i>Isothrix picta</i> .....		1			1						2	
<i>Phyllomys blainvillei</i> .....	1	2	50.0		1					1	3	33.3
<i>Phyllomys brasiliensis</i> .....		5									5	
<i>Rattus rattus frugivorus</i> .....		3			1						4	
<i>Rattus rattus rattus</i> .....		4			1						5	
<i>Nectomys squamipes</i> .....	8	137	5.8	5	80	6.3	5	74	6.8	18	291	6.2
<i>Oryzomys oniscus</i> .....	246	616	39.9	49	126	38.9	9	20	45.0	304	762	39.9
<i>Oryzomys eliurus</i> .....		5		8	104	7.7	1	4	25.0	9	113	8.0
<i>Oryzomys breviceps</i> .....	48	114	42.1	27	84	32.1	3	9	33.3	78	207	37.7
<i>Rhipidomys maculipes</i> .....		3		2	21	9.5		2		2	26	7.7
<i>Oecomys cinnamomeus</i> .....		2		5	9	55.6		1		5	12	41.7
<i>Akodon nigrita</i> .....	10	131	7.6	4	27	14.8				14	158	8.9
<i>Akodon arviculoides</i> .....		7		14	137	10.2	4	18	22.2	18	162	11.1
<i>Blarinomys breviceps</i> .....		4									4	
Total.....	313	1072	29.2	114	593	19.2	22	129	17.1	449	1794	25.0

two to three months later. On the other hand immunity in primates, as in humans, seems to be of very long duration, probably for life.

*Rodents:* Neutralization tests were performed on sera from 1,794 rodents of which 449 or 25 per cent were positive. All species in which the number examined was in excess of 10 contained one or more positive reactors, with the exception of *Proechimys setosus* (table 9). There was considerable variation in the percentage of positives among the different species. Among those tested in significant numbers *Oryzomys oniscus* and *Oryzomys breviceps* gave the highest percentage, 39.9 and 37.7 per cent respectively.

The percentage of positive reactors among the total specimens captured in old-type forests was greater than among those captured in young-type or swamp forests. However, this discrepancy may be attributed to the fact that the species which tend to give a high proportion of positives occur with greater frequency in the old forest type of vegetation. The results obtained with any given species, were essentially the same regardless of the site of capture.

If a positive neutralization test reflects an acquired specific immunity, the proportion of positive reactors would be expected to increase with the age of the animals, especially if the disease were endemic among them. This expected age distribution does not occur among the rodents examined; there was no

TABLE 10

## MARSUPIALS

Results of neutralization tests with yellow fever virus, with species listed according to type of vegetation in which they were captured

SPECIES	TYPE OF VEGETATION											
	Old-type forest			Young-type forest			Swamp forest			Grand Total		
	Pos.	Total	Per cent Pos.	Pos.	Total	Per cent Pos.	Pos.	Total	Per cent Pos.	Pos.	Total	Per cent Pos.
<i>Didelphis marsupialis</i> .....		152			56			19			227	
<i>Metachirops opossum</i> .....					1						1	
<i>Metachirus nudicaudatus</i> .....		187		1	59	1.7				1	246	0.4
<i>Monodelphis americana</i> .....		2			3						5	
<i>Marmosa cinerea</i> .....	3	46	6.5	6	63	9.5	1	5	20.0	10	114	8.8
<i>Marmosa agilis</i> .....	1	8	12.5		1		1	2	50.0	2	11	18.2
<i>Marmosa murina</i> .....		9		5	44	11.4		4		5	57	8.8
<i>Marmosa incana</i> .....		2			61		1	15	6.7	1	78	1.3
Total.....	4	406	2.2	12	288	4.2	3	45	6.7	19	739	2.6

essential difference in the positive reactors among the young, young-adults, and adults, the percentage being 24.8, 25.1 and 25.3 per cent respectively.

*Marsupials:* Only 19, or 2.6 per cent, of 739 marsupial sera examined gave a positive neutralization test. Fifteen of the 19 positives occurred among *Marmosa cinerea* and *Marmosa murina*. In each of these species the percentage of reactors was 8.8 per cent. The sera of two of 11 *Marmosa agilis* and one of 78 *Marmosa incana* neutralized yellow fever virus. None of the 227 *Didelphis marsupialis* and only one of 246 *Metachirus nudicaudatus* reacted positively (table 10).

Positive reactors were captured in all three types of forest. The highest percentage of positives occur among those derived from swamp forest, but the numbers involved are entirely too small to be significant.

*Primates:* Of the 1,851 primates shot or captured, a blood specimen was obtained and the test for the presence of serum antibodies against yellow fever virus was satisfactorily completed upon 1,711 (Table 11). The total group consisted of 1,694 *Callithrix penicillata*, 11 *Leontideus chrysopygus*, and 6 *Alouatta palliata*.

*xanthosternus*, 2 *Calicebus gigot*, and one *Alouatta guariba*. Ninety-nine or 5.8 per cent of the *C. penicillata* and one each of the *L. chrysomelas* and *Cebus xanthosternus* gave a positive neutralization test. The remainder were negative. Unsatisfactory or inconclusive tests were excluded.

For reasons previously mentioned, the sampling was uneven and many more marmosets were obtained from the northern part of the area than from the southern more extensively forested portion. Nevertheless, the captures served to give some indication of the distribution of immunity. As will be observed from map 5 immune marmosets (*C. penicillata*) were found widely distributed over the *Study Area*, although none was found north of the Rio Almada which flows just north of the collecting stations of Almada and Urucutuca.

The highest percentage (48.5 per cent) of positive reactors were encountered in the vicinity of the collecting station at Pirataquicé. At the Fortuna station

TABLE 11

*Results of neutralization tests with yellow fever virus on sera from Callithrix penicillata, listed according to type of vegetation where animals were captured*

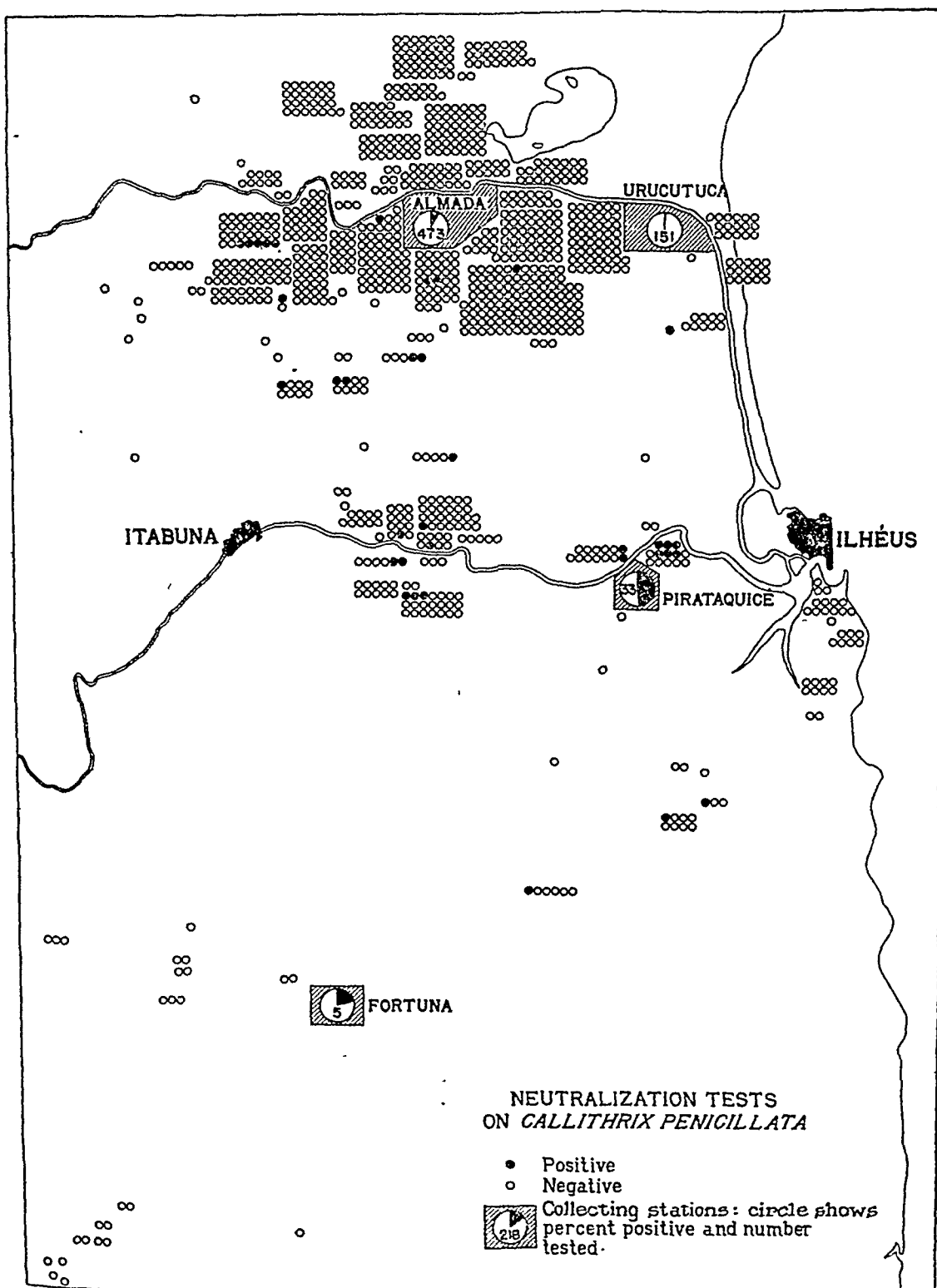
TYPE OF VEGETATION	NUMBER TESTED	NUMBER POSITIVE	PER CENT POSITIVE
Old-type forest.....	117	27	23.1
Young-type forest.....	558	25	4.5
Swamp forest.....	15	0	0.0
Cacao plantation.....	876	45	5.1
Banana patch.....	25	0	0.0
Coconut grove, etc.....	60	2	3.3
Not specified.....	43	0	0.0
Total.....	1694	99	5.8

Note: Among seventeen other primates tested for immunity, two proved to be immune; one was a *Leontocebus chrysomelas* and the other a *Cebus xanthosternus*. Both were captured in old-type forest.

only 5 *C. penicillata* were captured, one of which was immune. But in addition one of the two *Leontocebus chrysomelas* and a single specimen of *C. xanthosternus* captured in the vicinity were also immune, thus giving three immunes among the eight primates examined. Forty-four of the 473 (9.3 per cent) captured near the station at Almada and two of 151 (1.3 per cent) captured near the station at Urucutuca gave positive reactions.

The ratio of positive reactors among the *C. penicillata* is related to the type of vegetation in which they were shot or captured (table 11). A significantly higher percentage of immunes (23.1 per cent) derived from old-type forests than from any other environment. The two positive *Leontocebus chrysomelas* and the *Cebus xanthosternus* also came from forests of this type. While a few immunes were found in cacao plantations, young-type forests and coconut groves, the percentage is much lower than in old-type forests.

Analysis of the results of the neutralization test according to estimated age

MAP 5. Place of capture of marmosets (*C. penicillata*) and results of neutralization tests

of the marmosets revealed that seven of 180 (3.9 per cent) of "young" marmosets (less than six months of age), five of 319 (1.6 per cent) of "young-adults", and 85 of 1,138 (7.5 per cent) of "adults" were immune. The age was not entered on the records of 57 specimens of which two (3.5 per cent) gave a positive reaction. The somewhat, though not statistically significant, higher ratio of immunes among the young than among the young-adult animals suggested that a transient immunity may be passed from the mother to offspring. As is to be expected in acquired immunity, the highest rate was encountered in the adult or older animals.

*Other Orders of Mammals:* Of other orders of mammals, consisting of Edentata, Carnivora and Chiroptera, only two out of 130 blood specimens neutralized yellow fever virus. Among the Edentata, one *Dasipus novemcinctus* was positive, and a similar result was obtained with *Glossophaga soricina* of the order Chiroptera. The distribution of species tested in relation to the site of capture is listed in Table 12.

*Birds:* Blood samples for neutralization tests were obtained from 114 birds, comprising 41 species. Three of the blood specimens inactivated virus. These were from the following species: *Ceophloeus limatus*, *Cacicus cela* and *Saltator maximus*, the first two of which were shot at Pirataquicé and the third at Almada.

#### *Complement-Fixation Tests*

*Marmoset Sera:* Of 24 marmoset sera giving a positive neutralization test, four were found to fix complement in the presence of yellow fever antigen. Two of the animals from which these sera were obtained came from the vicinity of the collecting station at Almada and the other two from around Pirataquicé. One marmoset serum, which was toxic to mice and therefore could not be satisfactorily examined by the neutralization test, also gave a positive complement-fixation test. This serum came from an animal captured at Pirataquicé. Five other sera fixed complement in a dilution of 1:4. This is ordinarily considered to be a doubtful reaction, but with marmoset sera it may be significant. One of the latter sera came from an animal captured at Pirataquicé and the remaining four were from Almada and a neighboring fazenda. Five hundred and eighty-one sera that were negative by the neutralization test were negative for the complement-fixation test as well.

#### *Attempts to Isolate Virus*

*Mammals Other Than Primates:* Sera from a total of 1,756 mammals were inoculated into non-immune marmosets in search of yellow fever virus. These sera came from 579 Marsupialia, 1,123 Rodentia, three Carnivora, 50 Chiroptera and one Edentata (table 13). Most of the sera from animals belonging to the genus *Marmosa* were pooled before inoculation, but some of the sera from *Marmosa cinerea* were inoculated separately. Also sera from animals of the genera *Oryzomys*, *Oecomys* and *Rhipidomys* were pooled before inoculation, with the exception of a few sera from *Rhipidomys maculipes*. The marmosets which died during the series of inoculations or shortly thereafter were autopsied and,

unless it was obvious that they had succumbed from some intercurrent infection, liver suspensions were inoculated intracerebrally into a group of white mice. None of the surviving marmosets developed immunity to yellow fever, nor was the virus obtained from any of those which died during the course of the experiment. It is admitted that the method used was of doubtful value in

TABLE 12

Results of neutralization tests on sera from mammals of orders *Edentata*, *Carnivora* and *Chiroptera*, according to site of capture

	FORTUNA		PIRATA-QUICÉ		ALMADA		URUCUTUCA		OTHERS		TOTAL	
	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total	Pos.	Total
<i>Edentata</i>												
<i>Tamandua tetradactyla</i> .....									7		7	
<i>Scaepus torquatus</i> .....				5			1		2		8	
<i>Dasipus novemcinctus</i> .....			1	5			1		1	1	7	
Total.....			1	10			2		10	1	22	
<i>Carnivora</i>												
<i>Cerdocyon thous</i> .....				1	2				3		6	
<i>Tayra barbara</i> .....	1										1	
<i>Potos flavus</i> .....	3		14								17	
<i>Procyon cancrivorus</i> .....			1								1	
<i>Grison vittatus</i> .....									3		3	
Total.....		4		16		2			6		28	
<i>Chiroptera</i>												
<i>Glossophaga soricina</i> .....	7		2	1	11				4	1	24	
<i>Artibeus planirostris planirostris</i>									1		1	
<i>Lonchoglossa ecaudata</i> .....					2				2		4	
<i>Lonchophylla mordax</i> .....					4		1		3		8	
<i>Carollia perspicillata</i> .....	1		1		7		1		4		14	
<i>Molossus rufus</i> .....					7				2		9	
<i>Molossus obscurus</i> .....			1		1				5		7	
Not Classified.....			3		4		1		2		10	
<i>Sturnira lilium</i> .....			1								1	
<i>Dididurus albus</i> .....	2										2	
Total.....		10		8	1	36		3		23	1	80
Grand total.....		14	1	34	1	38		5		39	2	130

rodents since the virucidal action of a high percentage of the rodent sera probably would have prevented infection in marmosets, even though the virus might have been present in some of the sera in the pool. The same applies in a limited degree to the marsupials, although in a few instances only were sera from animals giving a negative neutralization test mixed with a serum that on subsequent examination was found to neutralize yellow fever virus.

TABLE 13

Sera from captured mammals inoculated into non-immune marmosets (*C. penicillata*) to determine presence of yellow fever virus

ORDER AND SPECIES OF MAMMALS	RESULT OF NEUTRALIZATION TEST ON SERA INOCULATED			TOTAL NUMBER OF SERA INOCU- LATED	MARMOSETS INOCULATED				
	Neg.	Unsatisfactory	Pos.		Number inocu- lated	Died*	Test for yellow fever virus	Survived	Neutralization test
<b>Marsupialia</b>									
<i>Didelphis marsupialis</i> .....	113			113	25	10	Neg.	15	Neg.
<i>Metachirus nudicaudatus</i> .....	161	5		166	25	10	Neg.	15	Neg.
<i>Marmosa cinerea</i> .....	69	3	11	83	13	7	Neg.	6	Neg.
<i>Marmosa agilis</i> .....	7	4	1	12					
<i>Marmosa cinerea</i> .....	32	1		33					
<i>Marmosa incana</i> .....	74	24		98	†25	9	Neg.	16	Neg.
<i>Marmosa murina</i> .....	49	21	4	74					
<b>Total</b> .....	505	58	16	579	88	36	Neg.	52	Neg.
<b>Rodentia</b>									
<i>Phyllomys blainvillei</i> .....	1	1		2	2		Neg.	2	Neg.
<i>Proechimys setosus</i> .....	22			22	6	2	Neg.	4	Neg.
<i>Nectomys squamipes</i> .....	203	4	15	222	23	8	Neg.	15	Neg.
<i>Isothrix picta</i> .....			1	1	1			1	Neg.
<i>Guerlinguetus ingrami</i> .....	1			1	1			1	Neg.
<i>Rhipidomys maculipes</i> .....	2	1		3	2	1	Neg.	1	Neg.
<i>Rattus rattus</i> .....	2			2	2		Neg.	2	Neg.
<i>Oryzomys oniscus</i> .....	376	31	232	639					
<i>Oryzomys sp.</i> .....	121	11	82	214					
<i>Oecomys cinnamomeus</i> .....	3	2	2	7	†33	13	Neg.	20	Neg.
<i>Rhipidomys maculipes</i> .....	8	1	1	10					
<b>Total</b> .....	739	51	333	1123	70	24	Neg.	46	Neg.
<b>Carnivora</b>									
<i>Griso vittatus</i> .....	1	2		3	1		Neg.	1	Neg.
<b>Chiroptera</b>									
<i>Molossus rufus</i> .....	8			8	1		Neg.	1	Neg.
<i>Glossophaga soricina</i> .....	24	13		37	4	1	Neg.	3	Neg.
<i>Artibeus planirostris</i> .....	1			1	1		Neg.	1	Neg.
<i>Lonchophylla mordax</i> .....		2		2	2		Neg.	2	Neg.
Not Classified.....	2			2	1	1	Neg.		
<b>Total</b> .....	35	15		50	9	2	Neg.	7	Neg.
<b>Edentata</b>									
<i>Dasypus novemcinctus</i> .....	1			1	1		Neg.	1	Neg.
<b>Grand total</b> .....	1281	126	349	1756	169	62	Neg.	107	Neg.

\* Died: Indicates marmosets that died before the period for testing for immunity.

† Sera pooled before inoculation.

*Marmosets:* Yellow fever virus was isolated from each of four marmosets that died shortly after arrival at the field laboratory at Pontal. The circumstances related to these isolations will be presented later. As has been indicated under "Field and Laboratory Methods," only marmosets which died shortly after capture (2 weeks) were examined for virus. However, from those which survived this period, a second sample of blood was withdrawn and the serum submitted to the neutralization test. None of those which gave a negative neutralization test when captured subsequently developed immunity, so it may be assumed that none of the surviving marmosets were infected at the time of capture.

*Birds:* Near the close of the study 141 birds representing 45 species were shot. Their livers were removed, as previously described, and tested for the presence of yellow fever virus. The results were uniformly negative.

*Mosquitoes:* The number of mosquitoes inoculated into monkeys and marmosets (*C. penicillata*) in search of virus amounted to 172,873. The classification and place of capture of these mosquitoes is shown in table 14. Besides captures at the regular collecting stations at Fortuna, Pirataquicé, Almada and Urucutuca, a few were made at Fazenda São Pedro situated in a region devoted largely to the cultivation of cacao, about 15 kilometers southwest of the Almada station, and at Fazenda Retiro located on the opposite side of the swamp from the Urucutuca station.

The largest number (80,444) fell into Group A: *Trichoprosopon*, *Wyeomyia*, *Phoniomyia*, *Limatus* and *Sabethes*; followed by Group B: (78,013) comprising *Psorophora* and *Aedes*; Group D: (9,119) *Haemagogus*; and Group C: (5,297) representing *Chagasia*, *Anopheles*, *Taeniorhynchus* and *Culex*. It was the original intention to inoculate *Aedes leucocelaenus* separately but this species was found in such limited numbers that it was decided to include it with the other *Aedes*.

Yellow fever virus was isolated on only one occasion following injection of suspensions of haemagogus mosquitoes caught at the Almada station. As this isolation synchronized with finding of the virus in marmosets, aspects of this episode will be presented later.

Another virus, immunologically unrelated to yellow fever, was isolated from a monkey that received inoculations from Group B mosquitoes captured at Pirataquicé. This neurotropic virus is being described in separate publications (74, 75).

*Ectoparasites:* The following tabulation shows the number of rodents and marsupials from which ectoparasites were collected and inoculated into non-immune marmosets:

ORDER	NO. OF ANIMALS FROM WHICH ECTOPARASITES WERE COLLECTED	NUMBER OF MARMOSETS INOCULATED	DIED BEFORE IMMUNITY TEST	TEST FOR VIRUS	SURVIVED AND TESTED FOR IMMUNITY	NEUTRAL- IZATION TEST
Rodentia.....	940	27	15	Negative	12	Negative
Marsupialia.....	161	30	16	Negative	14	Negative



TABLE 14

Number and classification of mosquitoes inoculated into rhesus monkeys and marmosets in attempt to isolate yellow fever virus

GROUPS BY GENUS OR SPECIES	OLD-TYPE FOREST		CACAO AND YOUNG-TYPE FOREST		SWAMP FOREST		TOTAL
	Fazenda Pirata-quicé	Rib. da Fortuna	Fazenda São Pedro	Fazenda Almada	Fazenda Retiro	Urucutuca	
A <i>Trichoprosopon</i> .....	3012	2004	207	22536	178	140	28107
<i>Wyomyia</i> .....	10088	5291	877	6328	405	1094	24083
<i>Phoniomyia</i> .....	2976	5349	51	1058	113	792	10339
<i>Limatus</i> .....	4928	2510	334	3256	69	43	11140
<i>Sabethes</i> .....	4264	1771	99	556	52	33	6775
Total Group A.....	25298	16925	1568	33734	817	2102	80444
B <i>Psorophora</i> .....	632	293					925
<i>Psorophora albipes</i> .....	1355	232	3	56050	41	3812	61493
<i>Psorophora ferox</i> .....	712	522	136	370	11	31	1782
<i>Psorophora lutzi</i> .....	129	48	19	57	2	162	417
<i>Psorophora cingulata</i> .....	57	16	43	181	29	20	346
<i>Aedes</i> .....	1559	273					1832
<i>Aedes fulvus</i> .....	395	55	69	1655	97	58	2329
<i>Aedes nubilus</i> .....	249	42	44	24	1		360
<i>Aedes scapularis</i> .....	198	21	15	373	8	753	1368
<i>Aedes serratus</i> .....	2357	593	569	3017	228	204	6968
<i>Aedes fluviatilis</i> .....	1						1
<i>Aedes terrens</i> .....	7	12					19
<i>Aedes fulvithorax</i> .....	9	24		6	1		40
<i>Aedes (Howardina) sp.</i> .....	6	17		10			33
<i>Aedes leucocelaenus</i> .....	49	39		12			100
Total Group B.....	7715	2187	898	61755	418	5040	78013
C <i>Chagasia</i> .....	18	3					21
<i>Chagasia fajardoi</i> .....	28	31					59
<i>Anopheles</i> .....	62	107					169
<i>Anopheles nimbus</i> .....	5			177	23	14	219
<i>Anopheles fluminensis</i> .....	2			4	2		8
<i>Anopheles intermedius</i> .....	10			200	9	20	239
<i>Anopheles mediopunctatus</i> .....	9		2	91	29	14	145
<i>Anopheles minor</i> .....					2		2
<i>Anopheles tarsimaculatus</i> group.....	1			28	7	232	268
<i>Anopheles cruzi</i> .....	2	8	2	1			13
<i>Anopheles bellator</i> .....	24	167		30		3	224
<i>Anopheles triannulatus</i> .....	1			4		8	13
<i>Taeniorhynchus</i> .....	272	10					282
<i>Taeniorhynchus indubitans</i> .....	2	12	10	20		21	65
<i>Taeniorhynchus chrysonotum</i> .....	19			513	190	209	931
<i>Taeniorhynchus fasciolata</i> .....	90	7	77	631	1062	214	2081
<i>Taeniorhynchus albicosta</i> .....						12	12
<i>Culex</i> .....	25	9	9	353	34	115	545
<i>Culex coronator</i> .....	1						1
Total Group C.....	571	354	100	2052	1358	862	5297
D <i>Haemagogus</i> .....	5551	2514	139	891	20	4	9119
Grand total.....	39135	21980	2705	98432	2613	8008	172873

As will be seen from the above record, virus was not obtained from any of the marmosets that died during the course of the experiment nor did any of the survivors develop immunity to yellow fever.

*The Almada Episode:* The circumstances surrounding the isolation of yellow fever virus from marmosets and haemagogus mosquitoes in the vicinity of Fazenda Almada deserve a more detailed description.

On June 7, 1944, a marmoset was captured in a small patch of young-type forest, approximately 3 hectares (about 7 acres) in extent, and was sent to the field laboratory on the following day. The animal appeared sick on arrival at the laboratory. Four hours after a blood sample had been taken from the heart, the marmoset died. On autopsy the gross appearance of the liver suggested yellow fever infection, and a 10 per cent suspension of liver tissue was inoculated intracerebrally into six white mice. One of the inoculated mice

TABLE 15

*Record of inoculation of a Rhesus monkey with haemagogus mosquitoes and tests for yellow fever virus and antibodies in its serum*

DATE	MOSQUITOES INOCULATED		RESULTS OF INOCULATION OF MONKEY SERUM INTO MICE AND TEST FOR IMMUNITY
	No.	Origin	
July 17.....	4	Mico forest	Test for virus negative
July 18.....	6	Lavapes forest	
July 19.....	6	Mico forest	
July 20.....	99	Lavapes forest	
July 21.....	8	Mico forest	Test for virus negative
July 22.....	19	Lavapes forest	
July 23.....	4	Mico forest	
July 24.....	34	Lavapes forest	
July 25.....	30	Lavapes forest	Test for virus negative
July 26.....	8	Mico forest	
July 29.....			
August 9.....			Test for immunity positive

became ill on the 9th day. Subsequent passage of the brain suspension from this mouse revealed the presence of yellow fever virus. The isolation of yellow fever virus from this marmoset and three others captured in the vicinity during the month of August has been reported by Laemmert and Ferreira (32).

On July 17, following identification of the virus, intensive studies were initiated in this locality and continued without interruption for four months. These studies consisted of capture of mosquitoes and animals, search for virus and tests for immunity among the captured animals. Thereafter collections were made at intervals of about 40 days until April 1945.

Yellow fever virus was obtained from haemagogus mosquitoes which had been captured either in the small forest (Mico forest) where the virus was first isolated from a marmoset, or in a larger forest (Lavapes forest) about one-half kilometer distant. In that mosquitoes captured in these two forests

were inoculated into the same monkey, usually on alternate days, it is impossible to determine from which of the two forests the infected mosquitoes came. A record of the inoculations and tests for virus and antibodies in the serum of the inoculated rhesus monkey is shown in table 15.

Yellow fever virus was again isolated from a marmoset captured on August 7th at a distance of approximately 3 kilometers from the site where the first "positive" marmoset was captured. This second positive marmoset was trapped in a cacao grove within 30 meters of a house. On August 10th and 13th marmosets containing yellow fever virus were captured at a site between the places where the first and second positive marmosets were taken. These two marmosets were trapped within 100 meters of one another and at about 500 meters from where the second marmoset containing virus was captured.

Although captures of marmosets and mosquitoes were continued, no further isolations of virus were made. During the months of March, April and May, prior to the first isolation, 48 marmosets were captured and examined. During the months of June, July and August when the virus was being found in marmosets and mosquitoes, 113 marmosets were examined and, as above indicated, virus was isolated from four of them. During the ensuing four months (September to December), 252 marmosets were captured and from January to April 1945 an additional 18. No virus was isolated from any of them nor did any of them become immune following capture.

The number of mosquitoes captured in this area and inoculated into monkeys amounted to 98,432. Most of them fell into Groups A and B (Table 13). It is of interest and perhaps of significance, especially in view of similar experiences of others, that the virus was identified only in the haemagogus mosquitoes although they constituted less than 1 per cent of the total mosquitoes tested. It should also be noted that the virus was found in this mosquito only during the period when it was being isolated from marmosets.

In July trapping of animals other than marmosets was initiated. No virus was isolated either from the ectoparasites or sera from 161 marsupials and from more than 300 rodents captured in the area.

Unfortunately there is little information on immunity among the animal population previous to the isolation of the virus. Only 48 marmosets were captured prior to the isolation of the virus in June, one of which was immune. This marmoset was captured in May. Subsequently efforts to trap marmosets in the region were intensified and from June to December 407 specimens were obtained, of which 42 or 10.3 per cent were immune. Table 16 shows the month of capture and the ratio of immunes.

While these data are not very satisfactory, they do show that the ratio of immunes is significantly higher among the marmosets captured after the month of July than among those previously captured (difference  $9 \pm 2.0$  per cent).

Six of 62 *Marmosa cinerea* and four of 29 *Marmosa murina* gave a positive neutralization test. However, there is no essential difference between the positive reactors among these two species captured in this locality and those

captured elsewhere. None of 35 *Didelphis marsupialis* and 34 *Metachirus nudicaudatus* reacted positively. Nor did the sera of rodents captured at this station inactivate yellow fever virus more frequently than was observed among those captured at the other stations.

Inquiry among local inhabitants failed to reveal the occurrence of any illness resembling yellow fever during the period when the isolations of virus were being made. About 7 months previously blood samples had been taken from

TABLE 16

*Marmosets captured near Almada between March 1944 and April 1945 and the percentage immune to yellow fever virus*

	NUMBER CAPTURED	NUMBER POSITIVE	PER CENT POSITIVE
March & April 1944.....	11	0	0
May.....	37	1	2.7
June.....	54	2	3.7
July.....	15	0	0
August.....	52	6	11.5
September.....	85	13	15.3
October.....	108	12	11.1
November.....	60	4	6.7
December.....	33	5	15.2
January to April 1945.....	18	1	5.6

TABLE 17

*Results of immunity tests in residents of Almada before and after isolation of yellow fever virus from Marmosets and mosquitoes captured nearby*

AGE GROUP	PRIOR TO ISOLATION OF VIRUS			AFTER ISOLATION OF VIRUS		
	Total	Positive	Per cent Positive	Total	Positive	Per cent Positive
0-4	0	0	0	9	0	0
5-9	19	0	0	7	0	0
10-14	27	0	0	13	2	15.4
15-19	14	3	21.4	6	2	33.3
20-29	11	1	9.1	6	0	0
30-39	0	0	0	7	3	42.9
40-49	1	1	100.0	8	4	50.0
50+	0	0	0	2	2	100.0
Total	72	5	6.9	58	13	22.4

a number of people in this community and examined for the presence of neutralizing antibodies. Most of these persons, however, were vaccinated against yellow fever shortly following the first immunity survey; only six escaped vaccination. These six persons were again tested some months following isolation of virus from marmosets and mosquitoes and one had become immune. This was a boy of 14 years who lived about 3 kilometers from where the nearest positive marmoset was trapped but he stated that he made frequent visits to the

locality where the positive animals were captured. In addition blood samples were taken from 58 persons in the neighborhood who had not been included in the first survey and who had not been vaccinated.

Table 17 reflects the result of immunity surveys before and after the isolation of virus.

While the ratio of immunes among the group surveyed following the epizootic of yellow fever in marmosets is higher than in the group examined prior to the epizootic, when the groups are broken down according to age the differences are not significant.

Eleven of the sera giving a positive neutralization test, taken after the isolation of the virus, were subjected to the complement-fixation test and 10 gave a positive reaction. This ratio of positive complement reactors is considerably higher than was observed elsewhere in the *Study Area* and suggests that the infections were of recent occurrence.

#### DISCUSSION

One of the classical methods of sifting evidence is by the process of elimination, but in this instance negative evidence is not necessarily exclusory. It is, therefore, preferable to review first the positive evidence with the object of forming an opinion as to whether or not it offers an acceptable explanation for what is known about the behavior of endemic jungle yellow fever.

The salient affirmative information collected during this study was: the presence of yellow fever virus in captured marmosets and in haemagogus mosquitoes; a positive correlation of immunity in marmosets with the habitat of old-type forests; a similar correlation of immunity in the human population with contact with old-type forests, and the greater prevalence of haemagogus mosquitoes also in this type of forests. The involvement of marmosets, both *C. penicillata* and *L. chrysomelas*, in the cyclic transmission of the virus was further supported in the laboratory by the alternate passage of the virus isolated (Almada strain) through each of these two species of marmosets and a mosquito vector (*A. aegypti*) (30). It was also shown that the species of *Haemagogus* (*sp. pegazzinii*) found in this region is capable of transmitting the virus under laboratory conditions (29). Indeed, the species of *Haemagogus* used by Bates and Roca-Garcia (27) in their transmission experiments in Colombia is taxonomically very closely related, probably the subspecies, which Kumm *et al* (63) have designated as *H. spegazzinii falco*. Thus, it may be said that the circle of incriminating evidence against the marmoset (*C. penicillata*) as a vertebrate host and *H. spegazzinii* as an insect vector is complete: the virus was found in each in nature; their ability to serve respectively as host and vector was demonstrated in the laboratory; and there is a relation in the immunity in captured marmosets and in the prevalence of haemagogus mosquitoes to immunity in the associated human population.

There is, in addition, some circumstantial evidence that conforms to this concept. It has been noted that haemagogus mosquitoes are more prevalent in the older-type of forest. They are also found principally in the upper vegetational levels and they tend to feed during the brighter, warmer hours of the

day. The marmosets are likewise arboreal inhabitants that spend most of their time in the higher foliage of the forest. While the habits of marmosets have not been thoroughly studied, it is a common observation that they are more active during the early morning and late evening, and have the custom of resting or napping on the high branches of the trees during the mid-day hours, a habit which would make them easy prey to *haemagogus* at a period when these mosquitoes are most actively engaged in the search of a blood meal. There is in tropical rain-forests a stratification of microclimates and a corresponding stratification of fauna and flora which Bates (76) has picturesquely compared to the varying depths of the ocean and its influence upon marine life. It would, therefore, appear that the setting and the scene of the drama of jungle yellow fever, in so far as the primates and *haemagogus* mosquitoes are concerned, are essentially in the canopy of the forest and that the infection of man or other terrestrial animals is accidental and adventitious.

All other information gathered was essentially negative in character. While a number of species of rodents gave a positive neutralization reaction, there is reason to believe that such reactions resulted from non-specific virucidal substances in their sera and are unrelated to infection with yellow fever virus. The species giving the highest number of positive reactions are highly resistant to experimental infection with yellow fever virus irrespective of the virucidal activity of the serum.

The marsupials cannot be so lightly dismissed since some of them, *Metachirus nudicaudatus* and members of the genera *Marmosa* and *Caluromys*, have been shown to be relatively susceptible to infection with yellow fever virus. Indeed it was found possible to pass the virus isolated from marmosets through either *M. nudicaudatus* or *Marmosa cinerea* in combination with *A. aegypti*. Yet the neutralization tests in the captured marsupials imply that they are infrequently infected in nature. Only one of 246 *Metachirus* gave a positive neutralization test and as 10 of 19 *Marmosa* sera that neutralized yellow fever virus also neutralized Japanese B virus, the specificity of the neutralization of yellow fever virus is to be questioned. Moreover, there was no relation of the incidence of immunity in *Marmosa* to the incidence of immunity among the associated marmosets and the human population. If the marsupials had been involved to any extent, one would have expected to find a larger proportion of them immune following the epizootic among marmosets which occurred in Almada than was observed. Indeed, if they had been proportionately infected, the immunity rate among them should have been much higher than among the marmosets, as laboratory experience indicates that more than half of the marmosets which become infected succumb, while the infection is rarely, if ever, fatal either to *Metachirus* or the *Marmosa*. If all of the infected marmosets had survived, the number of immunes should have been considerably greater than actually observed.

None of 227 *Didelphis marsupialis*, the species which Bugher *et al* (31) thought was involved in the cyclic passage of the virus in certain Colombian forests, gave a positive neutralization test.

Not only did the immunity tests fail to reveal any definite evidence against

the rodents and marsupials, but search for the virus in their sera, as well as in the ectoparasites collected from them, gave negative results. Negative evidence is obviously not conclusive but the absence of virus in the ectoparasites should be given some weight, as on ecological grounds ectoparasites are better adapted to transmission of the infection in marsupials, and especially in rodents, than is the *haemagogus* mosquito. These mammals are for the most part nocturnal and during the day usually remain secluded in darkened retreats where the photophilic *Haemagogus* is unlikely to enter. It is believed this statement is generally justified although it may be contended that *Metachirus* and *Marmosa* occasionally sleep during the day in places that would expose them to the bite of *Haemagogus*.

The rodents and the marsupials have been discussed at some length as these are the only two orders of mammals, besides the primates and Chiroptera, that occur in sufficient numbers to act as transient hosts in the maintenance of the virus in the forest. The collection of bats during the course of this study was not sufficient to warrant any deductions but, as before mentioned, laboratory experience has shown that the species which have been studied are very resistant to infection. The same partially applies to birds. None, so far, have been found susceptible. It is believed that the one positive neutralization test in a bat and the two obtained in birds should, in the absence of supporting evidence, be regarded as non-specific.

Concerning vectors of the virus, the only other mosquito (*A. leucocelaenus*), besides *Haemagogus*, which has been found infected in nature, and capable of transmitting the infection by bite, was present in such small numbers as to rule it out as a likely vector in the Ilhéus region. Of the other three species of *Aedes* that have been shown to be capable of transmitting the virus, *aegypti*, *scapularis* and *fluvialis*, only one (*scapularis*) was present in moderate numbers, but unlike the *Haemagogus* its favored habitat is near the ground level and its prevalence does not coincide with a high immunity ratio in humans and marmosets. Thus both affirmative (identification of the virus) and circumstantial evidence point to the *Haemagogus* mosquito as being the principal if not the sole vector in this region.

However, the question has been raised as to whether there may be some other, as yet unknown, cycle of the virus and that epizootics among primates, similar to infection in man, are only tangential or aberrant offshoots from a more permanent fundamental reservoir. Certainly this possibility cannot be denied. Yet to entertain such an hypothesis there should be either factual evidence to support it or adequate reasons presented for not accepting the mosquito-primate cycle as an explanation of what is known about the behavior of the jungle form of the disease. In the review of literature and in the preceding paragraphs, we have set forth evidence against other hosts and vectors which we hold to be essentially negative, with the possible exception of that, largely gained in the laboratory, on *Metachirus*, *Caluromys* and *Marmosa* as possible vertebrate hosts, and on some additional species of mosquitoes as vectors of the virus. Even if these marsupials do play a rôle, it is probably a minor one and does not profoundly affect the behavior of the virus in the forests. The data collected in this survey

give no indication that virus may be maintained in marsupials alone and, until more convincing evidence in this respect is supplied, we are not disposed to accept this concept. It is probable that on occasions other mosquitoes, particularly *A. leucocelaenus*, may act as vectors of the virus but both factual and circumstantial evidence implies that haemagogus mosquitoes are the principal vectors.

If the virus remained over a long period in any restricted locality there would be reason to suspect some vertebrate carrier or an independent arthropod cycle, but in this study, as well as in studies made elsewhere, it appears that the virus even in so-called endemic regions has the custom of migrating and shifting its location, probably by a process of direct extension. It therefore seems justified to postulate that the virus is maintained, and is thus endemic in certain types of tropical rain-forests, by virtue of wandering epidemics, or rather epizootics. Immune reactors among humans and marmosets imply that in times past the virus had invaded various localities in the *Study Area* but during the course of these observations its actual presence was verified only in a restricted area (Almada) although the capture of marmosets giving a positive complement-fixation test in the neighborhood of Pirataquicé suggests its recent presence in this locality. The forests must of necessity be rather extensive in order to furnish adequate fuel, i.e., non-immune primates, in order to remain endemic. The epizootic fortuitously encountered at Almada probably represented an excursion of the virus from its more permanent habitat in the older and more extensive forests. The fact that these epidemics come and vanish argue against, rather than in favor of, a carrier reservoir. This behavior also implies that the transient vertebrate host is not too rapidly replaced as would be the case with rodents and marsupials. It requires a longer period for primates to replenish a non-immune population.

From the aspect of time, as pointed out by Bugher *et al* (31), the mosquito rather than the vertebrate is the reservoir of the virus. All experience indicates that the virus remains, or at least circulates, in an animal for only a few days, while the mosquito once infected retains the virus and is capable of transmitting it throughout its entire life. Unfortunately we have no information on the span of life of sylvan mosquitoes, such as *Haemagogus*, under natural conditions.

The absence of immune reactors north of the Almada River in both the human population surveyed and in captured marmosets indicates that the river constituted an effective barrier to the spread of the virus, as the vegetation immediately north of the river does not markedly differ from that to the south. This apparent failure of the infection to cross the river implies that the host was not a winged vertebrate and that in this instance the mosquito vector did not fly northward over the river.

While these studies in an endemic area support the concept of the primate-mosquito cycle of the virus in the forests, they do not explain the rapid dissemination of the infection during the epidemic waves that have extended over southern Brazil, unless it be that under favorable conditions the mosquito vector may be wafted into air currents and borne for considerable distances.

In concluding this discussion the similarities and differences of what has been



revealed concerning the epidemiology of jungle yellow fever in East Africa and in Brazil will be briefly noted. The similarities are: in Africa as in Brazil all convincing evidence points to primates as the vertebrate hosts of the virus (77); the most likely vector in the interior of the forests in East Africa is a mosquito (*Aedes africanus*) whose favored habitat is in the upper foliage; and as in Brazil man seems to play no intrinsic part in the sylvan cycle of the virus. The essential differences are: *A. africanus* (78), unlike *Haemagogus*, is a crepuscule or night feeder and it is supposed that transmission of the infection to monkeys takes place at night when the animals are asleep rather than during the daytime; and in Africa man is rarely if ever infected in the forests, probably because he does not enter or remain in the forests after nightfall, while in Brazil human infection is definitely associated with visits to the forests. Human infections in East Africa seemingly result from a secondary cycle in which *Aedes simpsoni* (78, 79) functions as vector. This mosquito is a plant-axil breeder and is found principally along the edges of forests and about human habitations. Presumably *A. simpsoni* initially acquires the infection from marauding monkeys but thereafter the virus may be transmitted from man to man through the medium of this mosquito. Thus, there is resemblance of this extrinsic cycle to the introduction of a jungle strain into urban centers where *A. aegypti* exists, as has been noted in South America, although in the latter instances it is probable that the virus was introduced by a person infected in the forests rather than by *A. aegypti* being infected by monkeys.

#### SUMMARY AND CONCLUSIONS

This epidemiological study of jungle yellow fever made in a region in Brazil where the disease is endemic comprised: an immunity survey of the human population; a comparison of the personal habits and living environment of those who had been infected and those who had escaped infection; a systematic collection of animals and mosquitoes in selected locations and in different types of forest within the *Study Area*; testing for the presence of neutralizing antibodies against yellow fever virus in the sera of captured animals; and search for virus in mosquitoes, in ectoparasites and in the sera or livers of collected mammals and birds.

The immunity survey involved 1,183 persons, and a personal history was taken on 707 who were born and had continuously resided in the neighborhood. The number of mammals collected totaled 5,322 and suspensions from a total of 172,873 mosquitoes were inoculated in search of yellow fever virus.

It was found that immunity to yellow fever in the human population was associated with out-of-door occupation, contact with forests especially of the old type, living in the vicinity of these forests and travel by foot. However, when correction was made for visits to old-type forests, the significance of the other factors was neutralized. That is, the dominant factor favoring infection was personal contact with the forests, which likewise explains the higher ratio of immunes among males and in the age group above 15 years.

In the course of these studies yellow fever virus was isolated on four separate

occasions from marmosets (*C. penicillata*) during what appeared to be a transient epizootic among these animals. At the time of this epizootic, and in the same locality, the virus was also obtained from *H. spegazzinii*.

The highest ratio of immunes was found among marmosets captured in the old-type forests, and haemagogus mosquitoes were four times more numerous in these than in other types of forest. Thus, there was an association of immunity both in humans and in marmosets and the prevalence of haemagogus mosquitoes with the older climax type of tropical rain-forest.

A high percentage of sera from certain species of rodents neutralized yellow fever virus but it is believed that the neutralization was attributable to some non-specific virucidal substances in the sera rather than to specific immunity to yellow fever virus.

While *Metachirus nudicaudatus* and at least one species of *Marmosa* (*M. cinerea*) were found to be susceptible to infection with the yellow fever strain isolated from marmosets, neutralization tests on the sera of captured marsupials did not suggest that they were intimately involved in the cyclic passage of the strain in nature.

Efforts to identify the virus in the sera and in the ectoparasites of mammals other than primates, and in mosquitoes other than *Haemagogus*, were unsuccessful.

Reasons have been discussed for believing that the maintenance of the virus in the region studied may be explained by the primate-mosquito (*Haemagogus*) cycle.

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